The Journal of Laboratory and Clinical Medicine

VOL. VIII ST. LOUIS, MO., MAY, 1923 NO. 8

ORIGINAL ARTICLES

NEW POINTS IN PHYSIOLOGY OF DIGITALIS THERAPY*

BY ORVILLE HARRY BROWN, M.D. Ph.D., PHOENIX, ARIZ.

DIGITALIS has become more efficacious in my hands as the years of my practice have grown. The increased efficiency has been due to two factors, to wit: The use of larger doses than formerly and a better understanding of the physiologic indications for digitalis. Some of the data I present I believe not to be in the literature and original with me.

The advantage of larger—massive—doses of digitalis has been reported by various physicians. The late Dr. George Crandall of St. Louis was the first to teach me that the administration of digitalis should be in amounts necessary to get results rather than in conformity with dosage tables.

In the winter of 1910 I was caring for a physician's wife who had pneumonia. Her heart action was unsatisfactory to such an extent that it seemed that except for the free use of various emergency stimulants there were numerous occasions when she was near death. I had already given her digitalin, the use of which at that time was my custom, in $\frac{1}{20}$ grain doses at four-hour intervals for 48 hours or longer; but there was no definite response on the part of the heart to this amount of digitalin. The average dosage of digitalin was given as $\frac{1}{100}$ grain. I felt that giving five times the average dose of digitalin was near the limit of safety and feared to give more even though the indications for it were plain. Doctor Crandall was called in consultation and after examining the patient he advised digitalis. I told him that she had had repeated doses of digitalin each five times the ordinary dose; and he replied, "All right, give her twenty times the ordinary dose." We did. A fifth of a grain of digitalin worked results that seemed almost miraculous, and the smaller doses

*Received for publication, January 16, 1923.

of digitalin thereafter maintained the heart in a fairly normal state during the next five to seven days to the crisis of the pneumonia.

I gradually came to using tincture of digitalis, not specifying the preparation, in thirty drop doses at short or long intervals as indicated. In severe cases I may give an initial dose of a dram or even more; but I prefer to give not over a dram and repeat in a relatively short time if necessary. Not infrequently I give thirty drops three times a day for a number of days. Except in the unusual case thirty drops twice a day for a week or possibly for two weeks and then thirty drops once a day for a week to a few weeks will usually give the desired effect. After the heart has reached the state desired smaller doses will usually keep the heart in good condition; or the thirty drops may be administered three or less times per week.

The one development which calls urgently for digitalis is cardiac dilatation. The best indication for cardiac dilatation, in the absence of fluoroscopic examination, is the location of the apex beat. When the apex beat is definitely more than eight centimeters to the left of the medium line of the sternum, other causes for its dislocation being eliminated, there is usually some grade of dilatation of the heart. In subjects with small hearts there is certainly some dilatation at eight centimeters. The further out the apex beat is from normal the more urgent the demand for digitalis. It is just as important to know, too, if there is a dilatation of the right heart; but diagnosing this is more difficult.

In examining for dilatation one should use inspection, palpation, percussion, and auscultation, confirming the results of one examination by each of the others. In my own experiences auscultating for the point where the sounds of the heart first have a pronounced increase in intensity has proved to be the most reliable index in locating the borders of the heart—except in those cases where the apex beat is readily seen or definitely palpated. Cardiac murmurs are no criterion as to the needs of a heart for digitalis.

The musculature of a heart that is dilated receives less blood through its coronary arteries than does the muscle of the same heart when it is of normal size. Were a heart spherical a certain increase in its diameter would cause 3.1416 times as great an increase in its circumference. The ratio between the diameter and the circumference of a heart probably approximates that of a sphere. The coronary arteries, as a heart dilates, stretch in length approximately the same amount as the circumference of the heart is increased. An elastic tube stretched lengthwise decreases in diameter. I know of no law that would apply to the ratio of decrease in diameter with definite amounts of stretching in the length of elastic tubes, even of rubber, and certainly not of tubes of human tissue.

In order to establish some figures (by a method admittedly crude) as to what might be the decrease in coronary arteries stretched over a dilated heart I took the following:

A heart normally 100 mm. in diameter is dilated to 150 mm., and this is not an extreme case: the circumference would be increased from 314.16 mm. to perhaps 471.24 mm.; a heart normally 110 mm. in diameter is dilated to 140 mm.; the circumference would be increased from 345.57 mm. to 439.82

mm. Nine pieces of rubber tubing, some quite fresh and live and others old and relatively poor in elasticity, of varying diameters were stretched so that 314 mm. of the unstretched were brought to 471 and again so that 345 mm. of the unstretched tubes were brought to 440 mm. The length of the tube unstretched and stretched, the diameters of the tubes when unstretched and when stretched with percentages of decrease in diameter due to the stretching, with the averages, are shown in Tables I and II.

TABLE I

LENGTH UNSTRETCHED	LENGTH STRETCHED	DIAMETER UNSTRETCHED	DIAMETER STRETCHED	DECREASE IN MM.	PER CENT
314.16	471.24	10.5	8	2.5	23.7
"	"	9.5	7.5	2	21
"	"	7	5.5	1.5	21.4
"	"	6	4.75	1.25	20.8
"	"	5	4	1	20
"	"	4	3	1	25
"	"	4	3	1	25
"	"	4	3	1	25
"	"	3.5	2.5	1	28.5
"	"			Average	23.6

TABLE II

LENGTH UNSTRETCHED	LENGTH STRETCHED	DIAMETER UNSTRETCHED	DIAMETER STRETCHED	DECREASE IN MM.	PER CENT
345.57	439.82	10.5	9	1.5	14.3
"	"	9.5	8	1.5	15.8
"	"	7	6	1	14.3
"	"	6	5	1	16.7
"	"	5	4.25	.75	15
"	"	4	3.25	.75	19
"	"	4	3.50	.50	12.5
"	"	4	3.25	.75	19
"	"	3.50	3.	.50	14.3
			Average		15.6

A decrease of 23.6 per cent in the diameter of a tube (or blood vessel) causes a 41.6 per cent decrease in cross sectional area; a decrease of 15.6 per cent in the diameter causes a 28.7 per cent decrease in cross sectional area. Then too as the diameter of a tube or blood vessel decreases, the friction between the liquid, passing through the tube or blood vessel, and the wall increases.

To show that stretching of capillaries causes a lessened blood supply take hold of a fold of the skin and put it on a stretch and observe the resultant pallor. It takes but relatively little stretching to make a noticeable change in color.

Since the coronary arteries are variable in diameter in various parts of any one heart and in different hearts it is not possible to apply a formula to them with any degree of accuracy; but by the figures of the above paragraphs and tables it is evident that a heart, dilated to only a moderate extent, may so stretch its coronary arteries that they may carry perhaps only 50 per cent

or less of their normal quotient of blood. The musculature of a dilated heart, even of a mild grade, does its work then while in an undernourished state. In such a heart digitalis, if given in sufficient dosage and the heart's condition is not so severe that the heart is past response, should cause a return of the heart to normal size, and the coronary arteries to their full blood carrying capacity. Another point not to be ignored in this connection is that with a dilated heart there is a tendency for a fall in the blood pressure on which the coronary circulation depends and digitalis counteracts this.

The usual teaching regarding the use of digitalis in pneumonia is that it should be withheld until there is a definite indication for it as shown by a fall in blood pressure and an increase in pulse rate. In my own opinion this is wrong. The argument advanced in favor of withholding the digitalis until plainly indicated, is that giving it before there is evidence of heart failure is like whipping a tired horse before nearing the end of the journey. I say, however, that giving the digitalis is not like whipping a tired horse but like feeding him; and one should not wait until near the end of the journey to feed the horse. In pneumonia the right side of the heart is put to an unusual test doing more work than usual and, when there is a dilatation, the entire heart has less nourishment than it has for its ordinary work. Therefore dilatation should be anticipated and digitalis should be begun coincident with the first intimation that there is a pneumonia. I am not sure but what this same advice should apply to a greater or less degree to even the chronic lung infections. I have found digitalis to be of extreme benefit in many cases of asthma, of tuberculosis, and other infections.

Another class of patients to whom I make it a rule to administer digitalis is the preoperative. A few doses of digitalis before the patient takes the anesthetic may save much grief during and following the operation. One patient who has been operated twice in the past few years had no digitalis before her first operation which kept her on the table for about on hour; she was badly cyanosed during the latter part of the operation, her apex beat was well out from the normal position and her recuperation was slow; before the second operation she had digitalis, was on the table for two hours or longer, came through the operation in good color and condition and had a rapid convalescence. I know full well that other factors than the use of the digitalis in the one instance and the nonuse in the other may have been active in causing the difference in the results in the two operations but I believe that the digitalis deserves much of the credit for the excellent condition in the second operation which was not present in the first. I have had patients suddenly collapse under anesthetics, whom I now believe would not have if digitalis had been administered before the operation. In some cases it seems wise to give the patient digitalis immediately following the operation, while he is still asleep, or if awake, before anything can be taken by the stomach; in such cases I add a dram or more of the tincture to the proctoclysis solution.

Many persons complain of slight dyspnea on exertion; in most of these no organic heart trouble can be found and at the time of examination no cardiac dilatation exists. In such cases it is probable that there was undue cardiac

dilatation at the time of the exertion which would have been easily found if examination had been made at the time, but which has disappeared at the time of examination. The first case which attracted my attention to this phenomenon was a young baker who came to me complaining of pain in the cardiac area and dyspnea on Tuesday, Thursday and Saturday. I learned that on these days he was doing heavy work and finally concluded that he was producing a temporary heart strain by the heavy work. Digitalis gave him relief. To individuals subject to heart strain it is wise to have them take digitalis when such exertion is anticipated; if it is not anticipated and the exertion is performed then have them take the digitalis as soon after the exertion as possible. There are times when any individual might well take this advice with profit. The heart muscle does any work demanded of it provided it has been carefully and systematically trained up to that amount of work. The athlete, however, is the only class of individuals who systematically and carefully trains his heart for periods of sudden strain. Even he, I presume, might find a few doses of digitalis, preliminary to hard contests, advantageous.

SUMMARY

The heart is an organ which may be subjected to exertion for which it has not been properly trained. The serious effect of overexertion on the heart is dilatation. As a heart dilates the coronary arteries are stretched in length and narrowed in diameter with the consequence of a reduced blood carrying power which may easily reach 50 per cent or more. At such times, then, the heart is doing more work than ordinarily and with less than usual nourishment. A heart that is dilated or in danger of being dilated needs digitalis, and in amounts to overcome or prevent the dilatation. The amounts needed may be much more than what has commonly been considered the proper dosage.

HYPERGLYCEMIA—BASED UPON A STUDY OF 2000 BLOOD CHEMICAL ANALYSES*

By Henry M. Feinblatt, M.D., Brooklyn, N. Y.

THE present paper represents an attempt to correlate some of the clinical evidences with the blood chemical picture in patients showing hyperglycemia. At the outset it was hoped that two highly important questions might be answered. What conditions other than diabetes mellitus may an abnormally high blood sugar suggest? What is the relation between hyperglycemia and glycosuria; i. e., how high must the blood concentration be before sugar appears in the urine, and is this threshold constant? A study of the tabulation soon convinced me that the data presented therein could give no definite answer to these questions. Thirty-four of the hyperglycemic patients studied presented clinical evidences of diabetes mellitus; forty-seven were considered nondiabetic in spite of their hyperglycemia. In this latter series the diagnoses included a heterogeneous group, and, with a few exceptions, could not account for the blood sugar values attained.

The difficulty in reconciling the blood sugar readings with the results obtained from the examination of the urine arises largely from the fact that the two specimens are seldom taken simultaneously. Blood sugar values oscillate widely and rapidly. Today a reading of 1000 mg. per 100 c.c. may be obtained; a few days hence, the figure may be down to 250 mg. This fact would appear to explain in part some of the discrepancies noted in this respect.

The two leading questions of practical importance cannot be answered on the basis of the cases here studied, but incidentally the analysis brings to light some points of considerable interest.

Most of our knowledge of blood chemistry has been acquired during very recent years. Not so with the subject of blood sugar. Quantitative determinations were made by Claude Bernard quite some years ago. In his *Leçons sur le Diabète*[1] he described the method in detail. While the technic was laborious and 25 c.c. of blood were required, his results were apparently quite accurate. He reported the normal figure for man as 90 mg. per 100 c.c., which figure still stands.

As regards the more recent methods, the usefulness of Benedict's test appears to be vitiated by the fact that the readings seem to be falsely elevated when there is considerable retention of creatin and creatinin. Experimenting by adding known amounts of creatin and creatinin, I was able to satisfy myself that this objection does not apply to Folin's test. The great majority of sugar determinations here reported were made by Folin's method.

*From the Clinical Laboratory, Department of Internal Medicine, Long Island College Hospital, Brooklyn, N. Y.

Received for publication, January 3, 1923.

MATERIAL STUDIED

Out of 2000 routine blood sugar determinations taken in the wards of the Long Island College Hospital, there were separated all of those cases, regardless of diagnosis, which exhibited a reading in excess of 150 mg. per 100 c.c. Complete blood chemical analyses were made upon medical cases as a matter of routine. This scheme of mechanical winnowing must obviously yield results which are entirely free from the prejudices of the writer. Because of the large number of cases the findings should be fairly representative.

The data upon all of the hyperglycemic patients were tabulated, but because of its great length this table has been omitted. Altogether 81 patients yielded blood sugar readings in excess of 150 mg. per 100 c.c. of blood.

TABLE I.

SUMMARY OF CLINICAL DIAGNOSES IN THE 81 HYPERGLYCEMIC PATIENTS

Diabetes mellitus	34
Hyperthyroidism	3
Cerebral hemorrhage	3
Chronic glomerular nephritis	3
Menopause	3
Chronic bronchitis	3
Rheumatic fever	2
Neurosyphilis	2
Syphilitic aortitis	2
Miscellaneous conditions (occurring only once)	26

The outstanding features of this chart are (1) the finding of clinical diabetes mellitus in 42 per cent of the patients who showed hyperglycemia, and (2) the heterogeneous nature of the clinical diagnoses in the other group of 47 apparently nondiabetic individuals, representing 58 per cent. The presence of an abnormally high blood sugar concentration can be explained by the diagnosis in only a small percentage of the nondiabetics.

Various endocrinopathies, particularly hyperthyroidism, hyperpituitarism and hyperadrenalism, have repeatedly been demonstrated to exhibit hyperglycemia and glycosuria. Consequently, this finding in three cases of hyperthyroidism is not remarkable.

Glycosuria has frequently been observed in organic diseases affecting the brain, particularly in the region of the ventricles or the hypophysis. Claude Bernard in 1849, in his famous piqûre experiment, first demonstrated the influence which the nervous system may exert in the production of glycosuria. By puncturing a spot on the floor of the fourth ventricle, he was enabled to induce an artificial diabetes in the dog. Although this experiment has not been confirmed in man, Osler's case, seen with Reiss at the Friedrichshain, Berlin, of a woman with anomalous cerebral symptoms and diabetes, in whom at necropsy a cysticercus was found in the fourth ventricle, furnishes an analogy. Under the caption of brain lesions would fit our three cases of cerebral hemorrhage, the two of neurosyphilis, the one of epidemic encephalitis, and the one in which postmortem examination disclosed a dural tumor in the neighborhood of the pituitary body.

In three individuals the high sugar reading appeared to be attributable to chronic glomerular nephritis. The figures in these cases were 210,181, and 153 mg. per 100 c.c. respectively. The coexistence of moderate hyperglycemia with nitrogen retention is quite usual. The apparent sugar values as estimated by Benedict's method are raised by creatin and creatinin, giving false readings where there is considerable retention of these substances. In Folin's method the sugar readings are not influenced by the amount of creatin and creatinin present. A study of twenty-nine cases of uremia by the author[2] showed that abnormally high sugar values are frequently associated with nitrogen retention, but that this finding is far from constant; in fact, normal and subnormal sugar values are quite compatible with maximal grades of nitrogen retention. Even in cases in which the urea nitrogen rose to 375 mg. the sugar values were frequently within normal limits. In those cases in which the sugar content was above normal, this finding was apparently due to increased tolerance rather than to retention. The author[3] investigated 43 cases showing retention of creatinin. Here moderate hyperglycemia was frequent, but the figures bore no proportion to those for creatinin, and subnormal sugar values were often found together with high-grade creatininemia.

Folin[4] in 1920 discussed this phase in a Harvey lecture as follows:

"In this connection I would call attention to the highly peculiar fact that bloods from nephritics having very high urea retention give by our original method, as by all other sugar methods, abnormally high values for the blood sugar. There does not seem to be any tangible reason why such bloods should contain any more sugar than the blood of normal individuals. There is room for suspicion that in such bloods other materials than sugar play an important part, that similar products in smaller amounts are present in all blood, that all sugar values obtained are high and that the lowest sugar values obtained must still be regarded as maximum values. We know, of course, that creatine and creatinine tend to raise the apparent sugar value of blood by Benedict's method. These products, however, do not have any such effect in our method, yet by our original method we did obtain abnormally high sugar values in bloods containing 150 mg. or more of nonprotein nitrogen per 100 c.c. of blood."

One patient in the nondiabetic series was suffering from a manic-depressive psychosis, and in three the symptoms associated with the menopause were so serious as to require admission to the hospital. This leads up to what I now regard as the keynote of those obscure hyperglycemias, viz., the emotional factor.

Since the impressive researches of Cannon, the part which the emotions play in affecting sugar metabolism has been thoroughly appreciated. In one experiment, Cannon[5] bound twelve cats, in all of which the urine was sugar-free, to a comfortable holder, leaving the head loose. All of the animals developed a well-marked glycosuria, the average time being one hour and a half. In those cats which stood the test calmly, the sugar was late in appearing; whereas in the animals which were perturbed, early glycosuria developed. On the day following the experiment, the urine of all of the cats was sugar-free.

If hyperglycemia and glycosuria can be induced by frightening a cat or irritating a dog, or by subjecting a student to the mental strain of acting as a spectator in the football stadium, it is not strange that it should be found in hospital patients as a result of psychic factors. The clinical record makes no note of the mental perturbation caused by anxiety over the outcome of one's illness, or by a disturbing visitor, or the emotional upset resulting from exasperation with an orderly, yet these circumstances may exert a determining influence upon the blood sugar curve.

In the psychoses, particularly melancholia and depressive states, hyperglycemia is of high frequency. Kooy[6] in 1919 reported this condition in 19 cases of melancholia and 4 of neurasthenia and psychasthenia. He noted it less often in confusional insanities and marked emotional upsets, and occasionally in general paresis and dementia precox. Schwab[7] in 1922 studied blood sugar tolerance curves in neurologic conditions. He found a high frequency of abnormally elevated curves in the psychoses, particularly in melancholic and depressed states. The curves in themselves were not diagnostic of any particular psychosis, but merely appeared to be proportional to the degree of emotional disturbance. In the neuroses, increased blood-sugar values showed a tendency to be present in those individuals who were depressed and who were in the habit of worrying excessively.

Of 47 apparently nondiabetic individuals exhibiting hyperglycemia, the clinical record in 33 cases, or 70 per cent, could offer no adequate explanation. Some of these patients, of course, might actually have been bordering upon a diabetic state. This possibility cannot be ruled out without a careful and prolonged follow-up study. However, the findings obtained after a complete study in the hospital were not such as to justify the diagnosis of diabetes mellitus.

In one case, the diagnosis, prolapse of the uterus with cystocele, and the clinical record could in no way account for the high blood-sugar value, 400 mg. per 100 c.c., and the presence of a large amount of sugar in the urine. These findings were obtained on admission. The sugar promptly disappeared from the urine, and, eight days later, the blood sugar had receded to 142 mg.

An examination of the figures in the series of diabetic cases revealed marked and rapid fluctuations in sugar values. A blood-sugar reading applies only to the date on which the specimen is taken. It is remarkable to note how rapidly the blood sugar concentration falls under strict dietetic regimen. Today a reading of 1000 mg. per 100 c.c. may be obtained. Tomorrow, it may be 666 mg., and the following day, 333 mg. So far from handicapping us in our understanding of an individual case, this great liability of blood sugar level is of benefit to us. It enables us to gauge quite closely just how well the patient is handling his particular diet. To adjust the diet in the light of information obtained from frequent blood sugar determinations is to handle the case in the most intelligent manner possible.

The fact that hyperglycemia may result from emotional states necessitates caution in the interpretation of a blood sugar reading. Confusion may be obviated by keeping in mind the fact that, except in the psychoses where the

affective disturbance is more or less continuous, this type of hyperglycemia is transitory. Persistence is the criterion of its pathogenicity.

Failure to take fully into account the effects which psychic influences may have upon the blood sugar concentration may lead to false conclusions in the line of *post hoc, ergo propter hoc* logic. Frenkel-Tissot[8] in 1920 reported his studies upon young Austrian soldiers who had escaped from Italian prison camps and fled over the mountains back to Austria-Hungary. He concluded that altitude does not affect the blood sugar, but that the latter is elevated by sun baths and electric light. In view of the harrowing experiences through which these young men had just passed, it appears to me that emotional factors might have played no inconsiderable part in determining the results.

An interesting observation has been made as to the effect of a tropical climate upon the blood sugar. DeLangen and Schut[9] in 1918, from their extensive researches both in man and in animals, were enabled to demonstrate that the blood sugar value ranges from 30 to 75 per cent higher in Java than in Europe. Residence inland in the mountains reduced these figures, which, however, promptly returned to their former level when the subjects went back to the seashore.

RELATION BETWEEN HYPERGLYCEMIA AND GLYCOSURIA

The normal blood sugar value ranges from 90 to 110 mg. per 100 c.c. The concentration of sugar in the blood must rise considerably above the high normal figure before it will appear in the urine in such quantities as to be detectable by ordinary tests. It is very difficult to say exactly what this level is, because the blood sugar curve fluctuates so rapidly, and the urine cannot be obtained synchronously.

Claude Bernard[10] in 1877 gave the threshold figure as 0.3 to 0.35 per cent. Labbé[11] in 1920 gave it as 0.2 per cent. McCay[12] and his coworkers in 1919 came to the conclusion that the threshold stimulus of the kidney for sugar excretion varies widely in different individuals and in the same individual under different circumstances. They could find no parallelism or arithmetical proportion between the blood sugar and that found in the urine. They believed the normal threshold to lie somewhere in the neighborhood of 170 or 180 mg. per 100 c.c. In diabetics they found this threshold higher, and it rose progressively with the duration and increasing severity of the disease. Indeed, before death, blood sugar values often reached great heights without appearing in the urine. Cammidge[13] in 1920 studied 700 diabetics and could find no constant threshold value. Some patients with a permanently high blood sugar curve passed comparatively little in their urine; in others, normal or subnormal values in the blood were associated with frank glycosuria.

Our cases serve to emphasize the inconstancy of this relation between glycosuria and hyperglycemia. Thus, in one case, the blood sugar values ranged from 500 to 660 mg.; nevertheless two of the urinary reports were negative for sugar. Other cases are contained in the tabulation in which the blood sugar attained a level of 500 mg. and in which nevertheless there was no glycosuria. In only six of the forty-seven non-diabetic individuals was there a coexistent

glycosuria. Still the figures in a large proportion of these cases were far above the normal threshold. It is probable that in the majority of these patients the hyperglycemia was transitory. This might perhaps have been accompanied by a transient glycosuria, which was missed because the urine examined was not the urine secreted at that particular time. In others, the absence of sugar from the urine appears attributable to a high renal threshold.

SUMMARY

Out of 2000 routine blood sugar determinations taken in the wards of the Long Island College Hospital, 81 yielded readings in excess of 150 mg. per 100 c.c. Thirty-four of these individuals, or 42 per cent, presented clinical evidences of diabetes mellitus. The remaining 47 patients were considered nondiabetic. A prolonged followup study might, of course, have proved some of these individuals to be diabetic; but a complete study in the hospital did not yield enough data to justify such a diagnosis. The nondiabetic series furnished a heterogeneous group, and in only 14 of these individuals, or 30 per cent, could the diagnosis account for the hyperglycemia. It is believed that a single report of an abnormally high blood sugar value cannot be construed as pathologic, inasmuch as transient hyperglycemia may result from emotional disturbances. A study of the relation between hyperglycemia and glycosuria served to emphasize the fact that the renal blood sugar threshold varies in different individuals and in the same individual under different circumstances. In diabetes a high threshold value may exist, and this tends to increase in proportion to the severity of the disease.

REFERENCES

[1]Bernard, C.: Leçons sur le Diabète et la Glycogenèse Animale, 1877, p. 198.
[2]Feinblatt, H. M.: Uremia, An Analysis of Twenty-nine Cases, New York Med. Jour., awaiting publication.
[3]Feinblatt, H. M.: Creatininemia, Based upon a Study of 1500 Blood Chemical Analyses, Am. Jour. Med. Sc., awaiting publication.
[4]Folin, O.: Blood Analysis and its Applications, Harvey Lecture, delivered Feb. 28, 1920.
[5]Cannon, W. B.: Bodily Changes in Pain, Hunger, Fear, and Rage, 1915, pp. 66 to 80.
[6]Kooy, F. H.: Hyperglycemia in Mental Disorders, Brain, Oct. 1919, xlii, 214.
[7]Schwab, S. I.: Diagnostic Value of Blood Sugar Curves in Neurology, Arch Neurol. and Psychiat., Oct. 1922, viii, 401.
[8]Frenkel-Tissot, H. C.: Fluctuations of the Sugar Content of the Blood in Mountain Climate, Deutsch. Arch. f. klin. Med., Sept. 10, 1920, cxxxiii, 286.
[9]deLangen, C. D. and Schut, H.: Sugar Content of the Blood in the Tropics and Its Importance in Acclimitization, Mededeelingen van den Burg. Geneesk., Dienst, Batavia, 1918, No. 3, p. 1.
[10]Bernard, C.: Leçons sur le Diabète et la Glycogenèse Animale, 1877, p. 211.
[11]Labbé, M.: Le Diabète Sucré, 1920, p. 23.
[12]McCay, D. and others: Sugar of Blood and Sugar in Urine in Varying Conditions of Health in Bengali, Indian Jour. Med. Res., April, 1919, vi, 485.
[13]Cammidge, P. J.: Estimation of Sugar in Blood in Diagnosis and Treatment of Diabetes, Practitioner, Feb., 1920, civ, 114.

AN INVESTIGATION ON THE PICRAMIC ACID METHODS FOR BLOOD SUGAR*

By Henry C. Sweany and Ethel M. Johnson,† Chicago, Ill.

SINCE Lewis and Benedict[1] first described their picramic acid method for the analysis of blood sugar, there have arisen from time to time various objections to it. Several modifications of this method have been described that have, in some cases, greatly improved it, while in others no advantage has been shown over the original method. The chief criticism has been that the methods (original and modified) do not give consistent results, and often give results that are too high. In a recent work one of the authors (H. C. S.[2]) demonstrated the ease with which certain substances interfere with the reaction and suggested the possibility of a similar interference in the test as applied to blood.

It is the purpose of this work to check these methods and if possible demonstrate any fault or virtue present. It is not especially the results in normal subjects but in pathologic ones that are of real significance, for the results in the relatively normal cases are not exaggerated enough to be of serious import. In the pathologic specimens, however, in which unknown reducing bodies or substances, such as phenol, may be present in large amounts, the possibility of error is increasingly great. It is in the pathologic subject that an accurate knowledge of blood chemistry is most desired.

EXPERIMENTAL

After the standardization of the methods was completed it was planned to apply them (a) to a series of normal or nearly normal subjects, (b) to a series of pathologic subjects, including a series of animals after injections of various substances.

The more recent method of Benedict[3] and the Myers and Bailey[4] modification was selected as the most representative of the picramic acid methods. Along with them the two methods of Folin and Wu[5] were used, not so much for an absolute standard as for a comparative study. There is no reason why the Folin method may not give results that are too low as well as for the others to give results that are too high.

The technic of the new Benedict method is in brief as follows: Two c.c. of whole blood are laked with 2 c.c. of water and made up to 25 c.c. gradually and with gentle rotation with sodium picrate solution. This is filtered and to 8 c.c. of the filtrate is added 1 c.c. of 20 per cent sodium carbonate

*From the Laboratories of the Municipal Tuberculosis Sanitarium of the City of Chicago.
Received for publication January 24, 1923.

†As this work was nearing completion, this young investigator was taken with an illness that terminated in death. Her work was continued by Mr. Armada T. Weathers and Dr. K. Lucille McCluskey, to whom the surviving author acknowledges appreciation.

solution. After mixing, this is heated for ten minutes in a water-bath at 100° C. and read against a standard similarly prepared. The sodium picrate for this method is prepared by dissolving 36 gm. of purified picric acid in water, with the aid of exactly 16 gm. of sodium hydroxide, the solution is filtered, cooled and made up to 1000 c.c. It should have an acidity of 0.05 to 0.04 normal.

The Myers and Bailey method consists in laking 5 c.c. of blood with 10 c.c. of water and adding one-half a gram of picric acid; this is shaken thoroughly and diluted to a final volume of 25 c.c. with water. To 3 c.c. of the filtrate is added 1 c.c. of 20 per cent sodium carbonate solution the tube stoppered with cotton and heated for 15 minutes at 100° C. in a water-bath, cooled, diluted to 10 c.c. with water and read against a standard sugar solution similarly prepared.

The original Folin and Wu method is as follows: 10 c.c. of blood are laked with 70 c.c. of water, and to this 10 c.c. of 10 per cent sodium tungstate solution are added, followed by 10 c.c. of exactly ⅔ normal sulphuric acid. This is shaken and filtered; 2 c.c. of this filtrate are placed in a special constricted test tube, 2 c.c. of an alkaline copper solution are added and it is placed in a boiling water-bath for exactly 6 minutes, it is then cooled in a beaker of cold water, 1 c.c. of phospho-molybdic phospho-tungstic acid reagent is added, and then 5 c.c. of saturated sodium carbonate solution. This is diluted to 25 c.c. and allowed to stand 5 minutes and read against a standard similarly prepared.

Folin's new method is the same as the old, except that the tubes are taken from the water-bath and cooled in a beaker of water for 2 or 3 minutes without shaking. To this, 2 c.c. of the new molybdate reagent are added, allowed to stand 2 minutes, diluted to 25 c.c. and read against a standard similarly prepared.

In addition, a plan was used in which a definite quantity of sugar was added to each blood filtrate and the amount recovered was estimated. For example in the Benedict method, the sugar, or color-producing substance calculated as glucose, found in the 8 c.c. of blood filtrate which in turn represented 0.64 c.c. of blood, is, say, 0.6 mg.; the amount added is 0.5 mg. but the amount found by color comparison is, say, 1.2 mg. Therefore, 0.1 mg. is recovered in excess of the sugar originally present plus the added sugar. This is recorded in the table as a positive correction. The argument for this method of checking is that the same substances that will cause an augmentation or reduction of the sugar reading in the blood filtrate will have a like effect on the added sugar. This can only be true where the added sugar is present in small quantities, because the concentration of sugar has a varying effect on the reduction of alkaline copper or other such oxidizing mixtures.

A more rigid technic was used in the picramic acid methods, than was required by the methods as described. The reagents were added in the same sequence each time, with approximately the same time limit for each step in

the process. The water-bath was kept at exactly 100° C. by dissolving sufficient calcium chloride in the water, to compensate for any variation in pressure. After placing the tubes in the water-bath, the temperature was brought back to 100° C. as quickly as possible, by the use of accessory burners, because the tubes cooled the bath down from 3 to 6 degrees.

Accordingly, each determination was done in triplicate; to one tube of blood filtrate was added 0.25 mg. of sugar; to another 0.50 mg. of sugar, while the third was analyzed without the addition of sugar. The sugar was added in 0.1 c.c. of solution to the quantity of blood filtrates recommended in the respective methods. In the Folin methods the technic was slightly altered. One-half of the blood filtrate was used (i.e., 1 c.c.) and to this 0.05 mg. of sugar was added in 1 c.c. of solution, making a volume of 2 c.c. as called for in the method. The results obtained with this technic were checked with the original, i.e., 2 c.c. of blood filtrate were used for analysis to which was added 0.1 mg. of sugar in 0.1 c.c. of solution. Due to the fact that practically identical results were obtained by both methods, and the decreased amount of work required, the former technic was adopted.

The first group to be studied consisted of 33 patients who were selected at random from the ambulant patients of this institution. They were either incipient or moderately advanced cases of tuberculosis. So far as the known blood chemistry is concerned, they were normal. Complete chemical analyses were made for uric acid, urea nitrogen, nonprotein nitrogen, preformed creatinine, total creatinine, cholesterol and blood chlorides. In no case were any abnormalities observed, thus these results have been omitted. The results are averaged and tabulated in Table I.

TABLE I

AVERAGE OF THIRTY-THREE SAMPLES

BENEDICT METHOD	MYERS METHOD	OLD FOLIN METHOD	NEW FOLIN METHOD	AMOUNT OF SUGAR RECOVERED MORE OR LESS THAN 0.25 MG. ADDED TO BENEDICT TEST MULTIPLIED BY 100	AMOUNT OF SUGAR RECOVERED MORE OR LESS THAN 0.25 MG. ADDED TO MYERS TEST MULTIPLIED BY 100	AMOUNT OF SUGAR RECOVERED MORE OR LESS THAN 0.50 MG. ADDED TO BENEDICT TEST MULTIPLIED BY 100	AMOUNT OF SUGAR RECOVERED MORE OR LESS THAN 0.50 MG. ADDED TO MYERS TEST MULTIPLIED BY 100	AMOUNT OF SUGAR RECOVERED MORE OR LESS THAN 0.05 MG. ADDED TO THE NEW FOLIN TEST MULTIPLIED BY 1000
105.7	99.8	100.0	94.5	+4.8*	+4.2	+10.5	+7.6	−1.5
				−4.2	−4.3	−7.8	−6.7	
				+1.8	+0.7	+4.5	+1.1	

*This row represents an average of the plus readings; the next row an average of the minus readings; while the last is a general average of all.

The average results obtained on these 33 samples of blood show that these methods gave a figure of decreasing value in the order named:

Lewis and Benedict	105.7	mg.	sugar	per	100	c.c.	blood.
Folin and Wu method	100.0	"	"	"	"	"	"
Myers and Bailey method	99.8	"	"	"	"	"	"
Modified Folin and Wu method	94.5	"	"	"	"	"	"

The average results of the sugar determinations by these various methods on the blood filtrate before and after the addition of .25 mg. and .50 mg. of glucose are arranged in Table I under the proper heading.

The results obtained in the picramic acid methods when sugar was added were very erratic. A quantity sometimes greatly in excess and again less than the amount added, was indicated. The grand average, however, showed a plus recovery in the picramic acid method and a negative reading in the new Folin method. The recovery in the new Folin method though always low, was consistent and gave results more in accordance to theory.

In the next group a study was made on various pathologic bloods. These were obtained from private patients, patients from our institution and from the Cook County Hospital through the kind permission of Dr. Moody, Resident Pathologist, to whom we wish to acknowledge our appreciation. There are also added to this list results obtained on animals after injection of phenols.

Table II shows the results obtained.

The first three patients may be grouped together, as all had marked retention of urinary constituents with the attendant symptoms. The Folin tests are 30-90 per cent lower than the Benedict. Furthermore, by the picramic acid method the added sugar gave figures which indicated a recovery almost double in amount to that actually present. The next three patients (4, 5, and 6) were also nephritics having a retention of urinary constituents, but when sugar was added it was almost a complete recovery in patients 4 and 6 and only a 50 per cent recovery in patient 5. The next four patients were diabetics or of diabetic tendency. In Nos. 8 and 9 all the blood methods gave concordant results, but in No. 10 there was a gross discrepancy. This patient had a more or less mottled history including among other things, alcoholism, empyema, lung abscess, gastric ulcer, and far advanced pulmonary tuberculosis. Three weeks prior to death diabetes developed, with 7 per cent sugar in the urine. At autopsy all of these conditions were verified and in addition an aortitis and slight endocarditis. The blood chemistry analysis in addition to the results given in Table II was as follows, expressed in mg. per 100 c.c. of blood: total phenols, 4.3 mg.; total nitrogen 2995 mg.; fats, 467 mg.; amino-nitrogen 6.0 mg.; total creatinine 5.1 mg.; chlorides 430 mg.; inorganic phosphorus 2.5 mg.

The striking thing about this case is the divergence of the two types of blood sugar determinations. Duplicates were run on succeeding days to preclude the possibility of error. At the present time we have no explanation of the phenomenon. No. 11 was a patient having a profound jaundice from an "acute catarrhal" inflammation. No. 12 was a normal woman who developed an acute "toxemia" with constipation. Neither are unusual, but

TABLE II

NUMBER	INITIALS	CASE NO.	BENEDICT METHOD	MYERS METHOD	OLD FOLIN METHOD	NEW FOLIN METHOD	% INCREASE OR DECREASE OF TOTAL SUGAR, WHEN 0.25 MG. OF SUGAR IS ADDED TO BENEDICT TEST	% INCREASE OR DECREASE OF TOTAL SUGAR, WHEN 0.25 MG. OF SUGAR IS ADDED TO MYERS TEST	URIC ACID	NONPROTEIN N.	PHENOL	UREA N.	CREATININE	REMARKS
1.	M. E.	C. C. H. 812815	115.8	–	93.0	73.0	+49.0	–	2.6	–	–	39.7	2.0	Nephritis, hypertension, cardiac decompensation, ascites, impending coma
2.	C. C.	C. C. H. 814724	81.7	–	63.0	45.0	+48.0	–	2.7	41.2	–	–	1.5	Nephritis, luetic origin?
3.	A. M.	C. C. H. 812634	86.2	–	68.0	51.5	+68.0	–	3.4	– –	–	20.1	1.7	Chronic myocarditis, nephritis with hypertension
4.	M. M.	C. C. H. 778160	153.0	152.0	–	(110.0 (111.0	+5.7	+6.8	8.3	– –	–	97.5	7.5	"Syphilitic" nephritis
5.	Patient of Dr. F.	–	55.0	38.3		50.0	–19.0	–36.0	4.7	– –	–	24.4	3.0	Nephritis, hypertension, "double mitral," coma
6.	Foster	C. C. H.	103.7	100.1	–	80.0	+1.7	+9.4	5.8	– –	–	24.0	3.0	Nephritic
7.	F. O. T. 1 mo. earlier	–	92.0	96.0	(110.0 (222.0	84.0	+3.0	–5.4	(1.3 (4.1	– – – –	– –	19.5 14.8	1.8 1.5	"Prediabetic"(?)
8.	A. M.	C. C. H. 784195	375.0	400.0	–	411.0	+4.1	–0.3	2.2	– –	–	11.5	–	Diabetic

TABLE II—CONTINUED

NUMBER	INITIALS	CASE NO.	BENEDICT METHOD	MYERS METHOD	OLD FOLIN METHOD	NEW FOLIN METHOD	% INCREASE OR DECREASE OF TOTAL SUGAR, WHEN 0.25 MG. OF SUGAR IS ADDED TO BENEDICT TEST	% INCREASE OR DECREASE OF TOTAL SUGAR, WHEN 0.25 MG. OF SUGAR IS ADDED TO MYERS TEST	URIC ACID	NONPROTEIN N.	PHENOL	UREA N.	CREATININE	REMARKS
9.	L. G.	C. C. H. 783417	222.0	183.0	–	208.0	+13.7	+10.5	2.6	28.2	–	12.6	1.4	Diabetic
10.	M. P.	7024	483.0 497.0	594.0 636.0	180.0 175.0	210.0 200.0	–1.8	–	–	–	–	13.2	–	Alcoholic, with lung abscess, empyema, "TB" peptic ulcer, diabetes
11.	A. C.	–	126.0	–	–	95.0	–1.8	–	–	–	–	–	–	Catarrhal jaundice
12.	M. U.	–	168.0	–	–	–	+40.0	–	–	–	–	–	–	Normal case with "acute toxemia" and constipation
		Dog	97.3	126.0	61.5	53.5	+9.0	+10.0	3.6	–	–	–	–	Before Injection
		II	165.4	202.0	100.0	93.0	+40.0	+35.0	3.6	–	–	–	–	After Injection of 20c.c. of 2.5% phenol
		Dog III	123.4 136.0	152.4) 157.0)	100.0	93.0	+13.4	+6.0	2.6		4.3	–	6.1	Before Injection
			322.0 312.0	408.0) 432.0)	150.0	148.0	+26.4	+12.6	2.8		7.3	–	7.3	After Injection of 10c.c. of 10% cold potassium phenyl sulphate, following which dog died of shock. Blood was taken from heart after death

it demonstrates that even in such patients the picramic acid tests may be unreliable.

In Dog 2, 20 c.c. of 2.5 per cent phenol was injected before and after which blood was drawn for analysis. The results are all that was expected. In Dog 3, 10 c.c. of cold potassium phenyl sulphate were injected and the dog died, apparently of shock. The second bleeding was done from the heart after death. These results are similar to those obtained in Dog 2. In Dog 3, total phenols, uric acid, and creatinine were determined.

DISCUSSION

It is evident that there are serious discrepancies between the picramic acid methods on the one hand and the copper reduction methods on the other. These discrepancies are not so marked in normal subjects and even in certain pathologic ones. In other pathologic subjects, however, the difference is too great to pass by without an investigation of the cause. After a rather exhaustive study, we have not been able to account for all this variation. At one time it was thought that phenols might be the principal interfering factor, but in patient No. 10, Table II, in which the phenols and the other known chemical constituents of the blood were normal, there was still almost a trebling of results by the picramic acid methods over the Folin methods. This same thing was true in the experiments on Dog 3, Table II. It may be that under certain abnormal conditions some products are formed from glucose, or some other chemical constituents of the blood, that will react in the picramic acid methods to give additional color. In that event, there will be an actual usefulness for these methods in determining the "picrate reducing substances other than glucose." Because of the nonuniform results in certain mixtures, (for example where phenol is present), these results cannot be standardized until more is known of the reaction.

In uncomplicated diabetes, there is no exaggeration of results by the picramic acid methods. In certain patients with grave nephritis and retention of urinary constituents, there is an actual diminution in the results by the picramic acid methods over the others. In one patient the added sugar was only half recovered. Possibly a combination of substances was present that suppressed the normal color development. In the majority of patients with nephritis and retention of urinary substances, however, there is a great augmentation of results by the picramic acid methods.

The merits of the check method devised need not be discussed here. The picramic acid methods gave results that were erratic and usually high while the Folin methods gave results that were consistent though slightly low. Possibly the true sugar value is a little above the results obtained by the new Folin method. There may not be complete oxidation of the glucose or part may be oxidized by the oxygen of the air. The average added sugar recovered was 3 per cent lower than that added. For reliability, accuracy, and simplicity it is far superior to any of the picramic acid methods. The excess results by the picramic acid methods may indicate the presence of substances that are not strong enough reducing agents to reduce copper oxidizing mix-

tures, yet are able to reduce alkaline picrate solutions by acting directly or indirectly on the alkaline picrate.

SUMMARY

(1) It has been demonstrated that the picramic acid methods for the estimation of blood sugar are unreliable in certain pathologic conditions.

(2) It has also been established that the presence of phenol or potassium phenyl sulphate in blood produces a false sugar result when these methods are employed.

(3) From results obtained upon pathologic subjects and by the addition of sugar to blood filtrates it would seem to indicate that the modified method of Folin and Wu for the estimation of blood sugar possesses many advantages over any of the other methods studied.

(4) It is suggested that the picramic acid methods may be used to determine the presence in the blood of "reducing substances other than glucose."

BIBLIOGRAPHY

[1]Lewis, Robert C. and Benedict, Stanley R.: Jour. Biol. Chem., 1915, xx, 61.
[2]Sweany, H. C.: To be published in June issue.
[3]Benedict, Stanley R.: Jour. Biol. Chem., 1918, xxxiv, 203.
[4]Myers, Victor C. and Bailey, Cameron V.: Jour. Biol. Chem., 1916, xxiv, 147.
[5]Folin, O., and Wu, Hsien: Jour. Biol. Chem., 1919, xxxviii, 106; Jour. Biol. Chem., 1920, xli, 367.

THE PRESERVATION OF NATURAL COLORS IN GROSS SPECIMENS*

By Oskar Klotz, M.D., Sao Paulo, Brazil

IN recent years there has been a growing interest in medical museums, and they are being adapted more generally to the needs of student teaching. This is particularly true of the museums associated with the Department of Pathology, where it has been found that properly preserved specimens of pathologic lesions can be used to great advantage to instruct the students in the progressive changes which formerly were demonstrated only in the microscopic sections.

There are few schools where there is an autopsy clinic of sufficient proportion to rely upon the routine material to illustrate all the features of the diseases which are discussed in the various subdivisions of pathology. This is particularly true in the United States, and it is here that we find that the entire attitude to the pathologic museum has been revised. The museum is no longer a storehouse for rarities of disease or grotesque anomalies arising in clinical practice. The more modern museum deals with specimens illustrating the common diseases where the progressive processes of the tissue change can be studied in their macroscopic appearances. Thus at first sight it would appear that many museums have their shelves filled with duplicates, but on closer study we find that these similar specimens show variations in a common process, or show advancing stages of the disease. A well organized series of specimens showing the lesions in pneumonia, tuberculosis, appendicitis, cholecystitis, and in many other inflammatory processes, is invaluable for teaching purposes, not only within the Department of Pathology, but also for the clinical departments which are interested in the general exposition of their subject during the practical exercises. We know of no other method whereby human pathologic studies can be presented to the student in an equally convincing manner. In our own experience it has never seemed advisable to use this material during the didactic discussion of disease, but we have found it much more advantageous to link the study of the gross specimen at the time when the student was carrying on his histologic exercises. For the student to familiarize himself only with the macroscopic characters of inflammation without at the same time gaining a concrete idea of the general characters of the lesion from his inspection of gross specimens, seemed to us an incomplete and improper presentation of the subject. Illustrative specimens can be collected and prepared for almost every type of lesion with which we desire the student to familiarize himself.

Furthermore, we have found it extremely stimulating to the student to per-

*From the Instituto Anatomo-Pathologico, Faculdade de Medicina e Cirurgia de So Paulo. Sao Paulo, Brazil.

Received for publication, February 3, 1923.

mit him, during his course in bacteriology, to observe the lesions produced in various tissues by the different groups and types of microorganisms. During the course in bacteriology it is not the intention to attempt to teach the pathologic lesions of the disease, but rather to make his study more interesting and practical than is possible by utilizing the technic of bacteriology alone. The students who have been instructed in this manner during their course in bacteriology are much better prepared to enter upon their studies in pathology.

The use of the museum for study and instruction entails considerable work and attention upon the part of the instructing staff to properly correlate the museum and its materials with these other studies. It necessitates bringing the museum specimens to the student, not taking the student for instruction to the museum. This means the repeated handling of many specimens and their conveyance to the laboratories desiring their use. A certain number of specimens are injured in this transport, but I am convinced of its great value over the other method which converts the study of museum preparations into a separate course.

To arrange and accommodate a museum which shall serve this purpose, requires much attention on the part of the professor and, furthermore, also requires a cooperation with the clinical departments from whom this material is gained. Specimens, to serve the best purpose, must illustrate the lesion as it is found in life. Improperly prepared specimens which have altered their shape, their size, their color, are of little value for the type of teaching which I am here discussing. It is necessary for us to improve the technic of preservation and mounting so that the specimens are readily available and the student may thoroughly visualize the tissues as they appeared in the individual. There are many sides to the problem of properly presenting such material. Well organized museums are expensive, but when they have once been introduced into the program of instruction they well repay the initial outlay.

A few years ago we recommended a new fluid for the preservation of natural colors of animal tissues. This method, slightly simplified, we have still in use and can speak for the excellent results which it has given.

Upon coming to Brazil and beginning the nucleus of a museum in the Department of Pathology, I continued to make use of the fluid containing chloral hydrate. As I found, however, that chloral hydrate was an extremely expensive product in Brazil, I was forced to review the methods previously in use and to adapt one which would serve for a tropical climate as well as recommend itself for its cheapness.

The preservation of natural colors in tissues involves two processes: (1) the fixation of the tissues and color, and (2) the preservation for final storage. In the past all methods have required two or three separate fluids. The fixation of tissues consists in subjecting them to chemical solutions which will destroy the normal ferments of the tissues, kill the bacteria, and also render the colloids of the tissues insoluble. During fixation there should be very little alteration in the characters of the tissue, so that no distortion is brought about. Furthermore, during this process of fixation the color contained within the tissues should not be destroyed or so seriously altered that it cannot sub-

sequently be restored. In the Kaiserling method the fixation is dependent upon the use of 10 per cent formalin, which produces a good fixation of the tissues, but at the same time reduces the hemoglobin of the blood and of muscle to a brown color. If this reduction has not been carried too far, the color may again in large part be restored by immersing the specimens in alcohol. The danger of producing too great a reduction of the hemoglobin has always been one of the difficulties in this method. In the method which we have previously advocated, we permitted the fixation of the tissues and the fixation of the color to go on simultaneously without necessitating the reduction of the hemoglobin.

The proper fixation of the tissues is the most important stage in the preparation of gross specimens. We have found that good results are much more easily obtained when some care is given to the preparation of the fixing fluid. We have found, for instance, that one may avoid much alteration in the tissue structure if the basis of the fluid consists of an isotonic salt solution. Furthermore, we have found that the tissues at autopsy as well as those removed surgically are prone to become acid within a short time. To prevent the influence of this acid change, we have added a small quantity of sodium bicarbonate to the salt solution. Such solution, without the addition of other substances, will maintain the color and the characters of gross specimens for several days when placed in the ice-chest.

For the actual fixation of the tissues and the red blood cells we have found nothing which is better than formalin. A pure formalin must be used. This has been added in a concentration of from 3 to 5 per cent to the above isotonic solution. It is preferable to use the formalin in a relatively weak concentration for the purpose of penetration. The use of a 10 per cent formalin solution causes a rapid fixation of the outer surface of the specimen which subsequently will not permit the entrance of the solution into the depth of the tissues. An equally complete fixation is gained by a 3 or 5 per cent solution if the specimen is permitted to remain a little longer in the solution, and the weaker solutions have the advantage, furthermore, of not distorting the specimen.

Sodium chloride	8.5
Sodium bicarbonate	5.0
Formalin	30 to 50
Water	1000

The above forms the basis of the fixing fluids. To this then may be added the chemicals for the preservation of the natural colors. When chloral hydrate is used for this purpose, forty grams are added to the above mixture.

In place of chloral hydrate, we have found that carbon monoxide will serve equally well. The specimens are placed in a large jar containing 3000 c.c. of the above mixture. The jar is provided with an inlet near the base and an outlet at the top or in the tightly fitting cover. Ordinary illuminating gas is passed through the fluid after the specimens have been put into it. Usually the gas pressure is insufficient to flow into the bottom of the jar, so that an electric suction pump attached to the outlet, is necessary. The flow of gas is continued for 15 or 20 minutes. This is repeated each morning for 5 to 7 days.

Specimens which are not very fresh and those like spleen, which contain large quantities of blood, frequently discolor the fixing solutions, which then require renewal.

The specimens, before being placed in the fixing solutions, should be reduced to the desired sizes, and one must avoid placing too many specimens into the fixing jar. Best results are obtained when there is at least five times the quantity of fixing fluid, in relation to the quantity of the specimens to be treated.

As in the case with all the types of fixing fluids, good results are difficult to obtain when large masses of tissue are treated. For example it is almost impossible to obtain satisfactory color fixation by treating a whole liver or spleen or even lung. These organs must be cut, and preferably cut in slices.

The use of carbon monoxide for the preservation of color, gives rise to a beautiful bright red color which is quite stable and is not easily reduced by the formalin. The red blood cells do not lake, and within a few days the blood is well set in the blood vessels, capillaries, and tissues. Muscle hemoglobin likewise retains a good color.

As soon as a specimen is well fixed it should be immediately mounted in an air tight jar. For final mounting we are still using the chloral hydrate solution as we have described above. Moreover, we have found that in the preserving fluid the chloral hydrate need not exceed a two per cent solution.

We have in our possession specimens prepared by the gas fixation method, which are now somewhat over a year old. The color is well retained and resembles closely the results we obtain by the chloral hydrate method. The cheapness of the solution and the ease of preparing the specimen by the gas-fixation method recommends its use, particularly in laboratories where large numbers of specimens are constantly treated. One of the common faults committed by museum technicians is to overload the fixing solution with specimens. The above cheap solution can be removed frequently until the fixation is complete.

VENTRICULAR FIBRILLATION IN MAN WITH TEMPORARY CARDIAC RECOVERY*

By Frederick A. Willius, M.D., Rochester, Minn.

A STUDY of the exposed dog heart in which ventricular fibrillation is visualized has made possible the recognition of characteristic electrocardiograms.

The waves resulting from conduction of the impulse through the ventricles and from contraction are absent, being replaced by irregular and variable undulations, following each other so closely that no period of cardiac inactivity is evident. The ventricles during fibrillation reveal extremely rapid and irregular incoordinate movements of minute amplitude. The heart at first shrinks, then dilates and no longer possesses the power of expulsion.

It has been generally conceded that ventricular fibrillation in man is incompatible with life although D'Halluin[1] has observed spontaneous recovery in cats, and Nobel and Rothberger[2] have observed it in dogs. Robinson and Bredeck[3] were the first to obtain electrocardiograms in man, showing temporary cardiac recovery from this disorder of mechanism. The observations in this study were made on a patient during a syncopal attack, associated with ventricular fibrillation, thirty-four and a half hours preceding death. Unconsciousness lasted about ten seconds and was accompanied by ventricular fibrillation.

REPORT OF A CASE

Case A394327, a man, aged forty-four years, was admitted to the Kahler Hospital, October 20, 1922, complaining of marked shortness of breath, swelling of the legs and cough. His mother had died of heart disease. During the Spanish-American war, twenty-four years ago he had had rheumatic fever, but no subsequent recurrent attacks. Following this illness he had no special cardiac complaints except that he was unable to sleep lying on his left side because of the pounding of the heart. He was actively engaged in the practice of law and his daily routine was strenuous. November, 1921, he had an acute illness, said to be influenza, and two months later, complained of palpitation and shortness of breath on effort. At this time several infected teeth were extracted. A month later severe pain developed in the right upper abdomen which persisted for about five weeks. No fever was noted during this time. Two months before examination in the Clinic the shortness of breath had increased, the patient was unable to lie down, his lower extremities swelled, and he coughed severely.

Physical examination revealed a thin man, evidently critically ill. There was marked dyspnea and he was unable to recline. The face was pasty and slightly edematous, and a distinct cyanosis was present. The heart was enlarged, the dullness extending 5.0 cm. to the right and 12.0 cm. to the left of the middle sternal line. The rhythm was irregularly interrupted by premature contractions. There were blowing systolic and diastolic murmurs at the apex, the former being well transmitted into the axilla. The pulmonic second tone

*From the Section on Medicine, Mayo Clinic, Rochester, Minn.
Received for publication, January 8, 1923.

was accentuated. The pulse rate was 122 each minute. Coarse, moist râles were heard over both lung bases posteriorly. The liver was not palpable. There was slight edema of the feet. The systolic blood pressure was 98, the diastolic 68. The urine contained a moderate amount of albumin and an occasional hyalin cast. Roentgenographic examination of the chest showed the heart to be markedly enlarged and there was infiltration of the lower right lobe which was thought to be due to congestion. The temperature was normal, or subnormal, throughout the illness.

SUBSEQUENT COURSE AND TREATMENT

Twenty-two c.c. of Minnesota leaf tincture of digitalis was given in four days. Fluids were restricted to 800 c.c. in twenty-four hours and the patient placed on a light protein and salt-free diet. Digitalis was given intermittently.

The patient showed no response to rest and treatment; all the symptoms became exaggerated and were accompanied by marked restlessness, requiring frequent opiates.

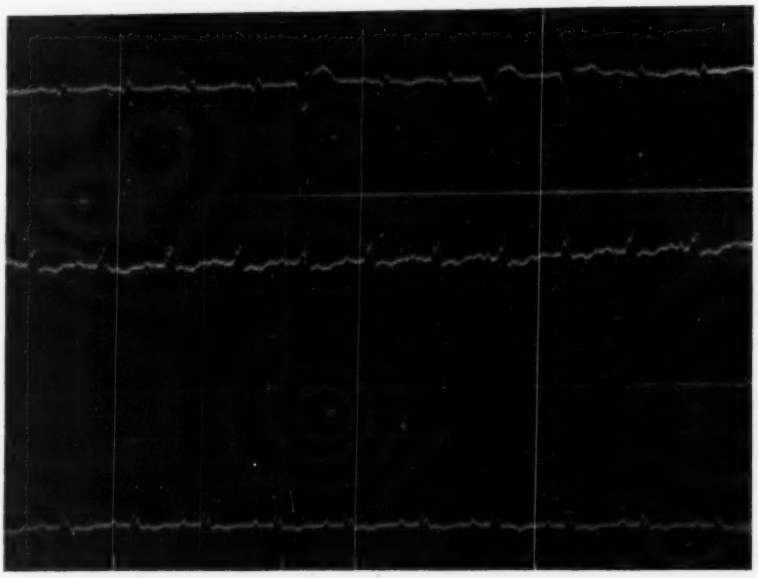

Fig. 1.

November 2 drowsiness was noted and while no appreciable qualitative change in the urine was evident, the amount was less, only 200 c.c. having been excreted the previous day. The blood urea was found to be 68 mg. and the urea nitrogen 31.7 mg. for each 100 c.c.

November 6 the drowsiness was more marked and the urea had increased to 144 mg. and the urea nitrogen to 67 mg. for each 100 c.c. On this day expectoration of bright red blood occurred, but there was no complaint of chest pain. During the course of electrocardiographic examination the patient suddenly became unconscious, there was no perceptible pulse at the wrist, cyanosis became quite profound and death seemed imminent. This attack lasted about ten seconds, after which the patient was much weaker,

and death occurred thirty-four and a half hours later. There was no repetition of syncope.

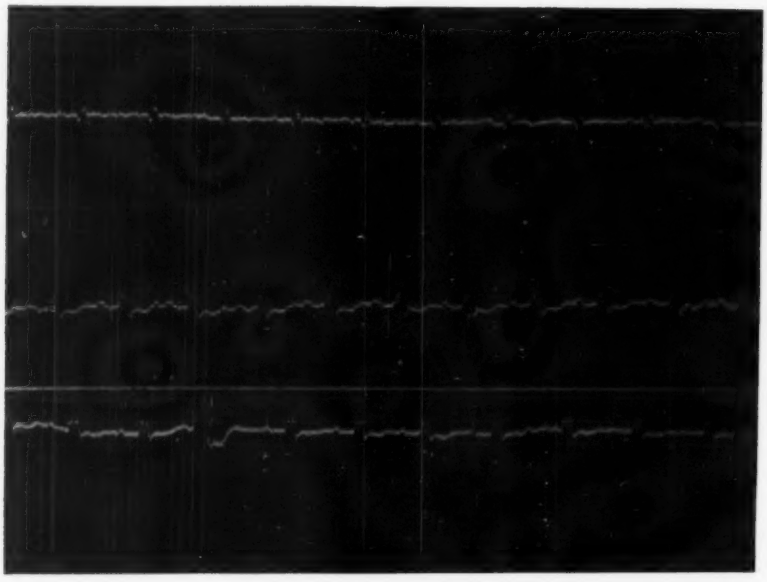

Fig. 2.

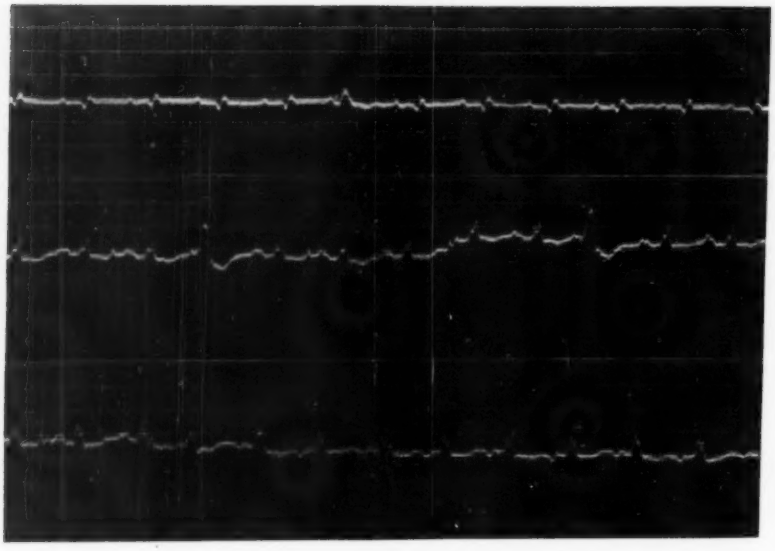

Fig. 3.

ELECTROCARDIOGRAMS

The first electrocardiogram (Fig. 1), taken the second day after admission, showed a rate of 100, and premature contractions of ventricular origin. The QRS complexes are aberrant in all derivations, being definitely notched. The T wave is negative in all derivations. No other abnormalities exist.

The second record (Fig. 2), obtained November 1, shows no appreciable change.

The third record (Fig. 3) was taken November 3 and besides the features

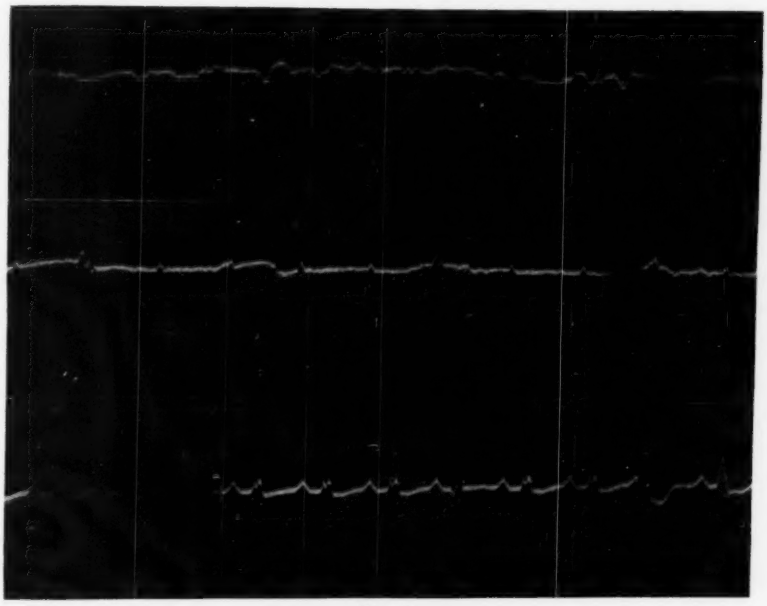

Fig. 4.

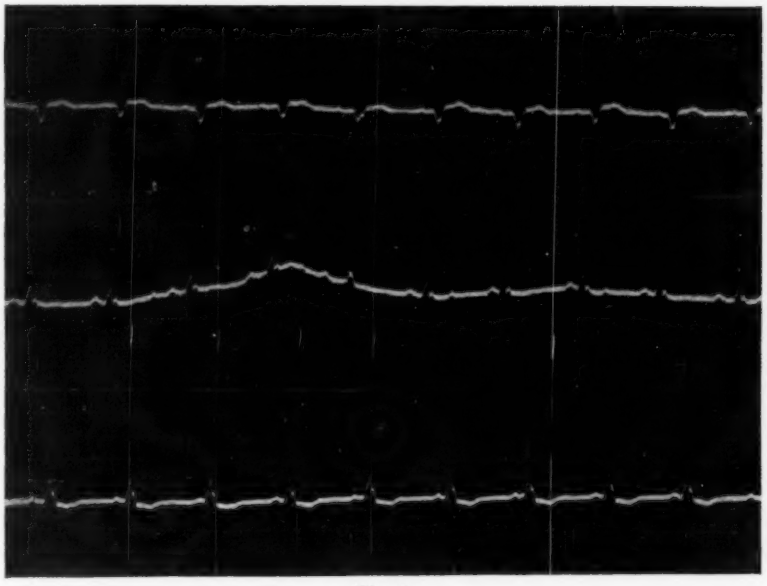

Fig. 5.

noted in the previous electrocardiograms, notching of the P wave in Derivation II and negativity of the P wave in Derivation III are present.

The fourth record (Fig. 4), taken November 6, shows ventricular fibrillation in Derivation I and was coincident with the syncopal attack previously

described. By the time that Derivation II was recorded the preexisting cardiac mechanism was reestablished. Unfortunately the transitional record was not obtained.

The last record (Fig. 5) was obtained November 7, eleven and a half hours before death. The QRS complexes are aberrant in all derivations and directed downward in Derivation I.

NECROPSY

The heart was enormously enlarged, principally as a result of dilatation. The weight of the organ was 610 grams. There was extensive myocarditis, practically all muscle fibers being involved. A low grade chronic endocarditis of the aortic and mitral valves was present. There was a mural thrombosis of the right auricular appendage with multiple infarcts of both lungs. The liver showed chronic passive congestion with marked atrophy. The spleen, kidneys, intestines and lungs were greatly congested. The kidneys were otherwise normal. A terminal bronchopneumonia was also present.

Histologic studies of the myocardium revealed, besides the extensive muscle degeneration, an occasional Aschoff body.

The extensive myocardial damage found at necropsy is a very tangible explanation of the ventricular fibrillation. The extreme dilatation of both ventricles further favored the development of this abnormal mechanism.

SUMMARY

A case is presented in which the patient showed progressive cardiac failure. During the syncopal attack fibrillation of the ventricles occurred. The patient lived thirty-four and a half hours after the attack. This case is the second instance of temporary cardiac recovery in man observed following ventricular fibrillation.

BIBLIOGRAPHY

[1]D'Halluin, M.: Le massage du coeur. Presse méd., 1904, xii, 345-349.
[2]Nobel, C. and Rothberger, C. J.: Quoted by Levy, A. G.: Massage of the Fibrillating Ventricles, Heart, 1920, vii, 175-190.
[3]Robinson, G. C. and Bredeck, J. F.: Ventricular Fibrillation in Man with Cardiac Recovery. Arch. Int. Med., 1917, xx, 725-739.

DUODENECTOMY, REPORT OF EXPERIMENT FOUR YEARS AFTER OPERATION*

By Frank C. Mann, M.D., and Kyoichi Kawamura, M.D., Rochester, Minn.

IN two previous publications we have described the technic for the removal of the duodenum and recorded the general results following such operation on several species of animals. We shall report here one experiment in which the animal (dog) was in good condition four years after duodenectomy.

Protocol.—December 11, 1918. Experiment 892. Dog C878, a male mongrel shepherd, weighing 20.7 kg., was placed under ether anesthesia and the duodenum removed at a single operation by the method previously described. The animal recovered from the operation and was kept under observation for four years. During this time the animal remained in excellent condition. It was weighed at intervals, its weight usually fluctuating between 19 and 21 kg. During the early part of the winter of 1920, it became slightly thin, its weight decreasing to 16.4 kg., the lowest recorded during the four years. Since 1920 it had not weighed less than 19 kg. The animal was always active, his appetite good and general condition excellent. It appeared perfectly normal, and no effects of the operation were observed. It was photographed several times, and the pictures compared. No difference was discernible.

December, 1922, four years after the operation, the animal weighed 20.5 kg. and was in excellent condition. It was killed by over-etherization, and necropsy was performed immediately. The animal was fat and the muscles were well developed. The site of abdominal incision was marked by a faint, hardly discernible scar. In the abdominal cavity the main evidence of a previous operation was a few adhesions of omentum to the site of the anastomosis of the jejunum to the stomach. The pancreas was small, weighing 12 gm., hard on palpation, but normal in general appearance. Both ducts were dilated, the major measuring 3 mm. in diameter. The liver was normal in color and size, weighing 500 gm. The organ cut with more than normal resistance and gave other evidence of increased connective tissue formation. The common bile duct measured 6 mm. in diameter and the hepatic ducts were also dilated. The gall bladder was shrunken to about one-third its normal size. The walls were thickened and pale. On palpation the gall bladder felt hard; it was opened and the contents were found to be white, thick and viscid with almost no odor. The mucosa was thickened and pale. The gastric and intestinal mucosae were normal in appearance. All other organs were grossly normal.

Microscopic examination revealed that all organs were normal except the liver, pancreas and gall bladder. The liver had a slightly increased

*From the Division of Experimental Surgery and Pathology, Mayo Clinic, Rochester, Minn.
Received for publication Dec. 16, 1922.

amount of perilobular connective tissue with round-cell infiltration in some areas. The walls of the bile ducts were thickened. On the whole, the organ was practically normal. The pancreas was normal except for an increase in the connective tissue around the ducts. The gall bladder showed the greatest changes. The walls were thickened with an increased connective tissue, and there was a large amount of lymphocytic infiltration throughout their entire thickness, particularly marked just under the mucosa. The mucosa was thickened and the cells enlarged.

SUMMARY

The duodenum was removed from a dog and the continuity of the gastrointestinal tract maintained by an end-to-end anastomosis of jejunum to stomach. The first portion of the jejunum thus assumed the position normally occupied by the duodenum. The common bile duct and pancreatic ducts were transplanted into this transposed portion of the jejunum at approximately the same distance from the pylorus and from each other as they occurred normally.

The experiment is of interest, (1) in showing the effect of removal of the duodenum; and (2) the effect of transplantation of the common bile and pancreatic ducts. The animal remained in perfect health and maintained its normal weight for four years following removal of the duodenum, and there is no reason to believe that the duodenectomy would ever have affected the health of the animal, had it been allowed to live longer. The experiment therefore definitely proves that in the dog the duodenum is not essential to life or good health, and that whatever function it may have can be compensated for by the remainder of the intestinal tract. It is also shown that the transplantation of the bile and pancreatic ducts can be carried out successfully, so that the respective glands will remain practically normal for a long time.

The conclusion made by Dragstedt et al, Grey, Moorhead and Landes, and by us, regarding duodenectomy, based on relatively short periods of observation, is corroborated by this experiment based on a long period.

BIBLIOGRAPHY

[1]Dragstedt, L. R., Dragstedt, C. A., McClintock, J. T. and Chase, C. S.: Extirpation of the Duodenum, Am. Jour. Physiol., 1918, xlvi, 584-590.

[2]Grey, E. G.: Duodenectomy—Its Effect Upon the Life of an Animal, Transplantation of the Pancreatic Duct. Surg. Gynec. and Obst., 1919, xxviii, 36-42.

[3]Mann, F. C. and Kawamura, K.: An Experimental Study of the Effects of Duodenectomy. A Preliminary Report, Jour. Am. Med. Assn., 1919, lxxiii, 878-880.

[4]Mann, F. C., and Kawamura, K.: Duodenectomy (An Experimental Study), Ann. Surg., 1922, lxxv, 208-220.

[5]Moorhead, J. J., and Landes, H. E.: Duodenectomy. A New Method. Jour. Am. Med. Assn., 1919, lxxii, 1127-1128.

CLINICAL AND LABORATORY PROCEDURES IN PEDIATRICS

By A. Levinson, M.D., Chicago, Ill.

(*Continued from page 439.*)

Blood Chemistry

THE chemical determinations on blood of clinical value are: sugar, nonprotein nitrogen, urea nitrogen, uric acid, creatinine, cholesterol, chlorides calcium and phosphorus. Of these, the first five mentioned are most important.

The most commonly employed system of blood analysis is that of Folin and Wu. Because of the rapidity of the methods and the small amount of blood needed in this system of analysis, it seems preferable to other methods. I shall therefore describe it here as used with slight modification at the Michael Reese Hospital chemical laboratories. For detailed accounts one should refer to the original work of Folin and Wu. It should be remembered, however, that because of the small amount of material needed and because of the personal factor entering into the reading of colorimeters, a slight inaccuracy in the method may mean a large percentage of error in the final results.

Removal of Proteins.—In determining sugar, nonprotein nitrogen, urea, uric acid and creatinine in the blood, the protein must first be coagulated. The following method is used: To a measured quantity of oxalated blood, (2 to 8 c.c. depending on the determinations desired) are added seven volumes of water, one volume of 10 per cent sodium tungstate, and one volume of 2/3 normal sulphuric acid, the latter to be added slowly from a burette while shaking the flask. The mixture is allowed to stand for at least five minutes, to coagulate the proteins. The color of the precipitate should now change from red to dark brown or chocolate. If the change in color does not occur, the blood has not been coagulated completely, most likely because too much oxalate has been used in obtaining the specimen. In such cases, 10 per cent sulphuric acid should be added drop by drop and the coagulum shaken after each drop until the color changes to chocolate. The mixture is now filtered through a large, dry filter. The clear, colorless filtrate is used for the various examinations. If the filtrate is to stand 24 hours or longer before examination, a drop or two of toluol should be added.

Determination of Blood Sugar.—Into a Folin sugar tube (pyrex), graduated at 25 c.c. (Fig. 14) should be introduced 2 c.c. of blood filtrate and 2 c.c. of alkaline copper tartrate solution.[1] Into another tube should be introduced

[1] *Alkaline Copper Tartrate Solution.* Forty grams of anhydrous sodium carbonate is dissolved in about 400 c.c. of water. This is transferred to a one liter volumetric flask. Now 7.5 grams of tartaric acid are added. When dissolved, 4.5 grams of crystallized copper sulphate are added, mixed and made up to the mark.

2 c.c. of a standard sugar solution[2] containing 0.1 mg. of glucose per c.c., and 2 c.c. of alkaline copper tartrate. The standard and unknown are heated together in boiling water for six minutes, after which they are placed in cold water for two minutes. To each tube is then added 2 c.c. of molybdate phosphate solution[3] and water is added after two minutes to the 25 c.c. mark. The solutions are mixed well and after standing for at least five minutes the unknown is read against the standard in the colorimeter setting the standard at 20.

The sugar is calculated from the formula:

$$\frac{20 \times 100}{\text{Reading}} = \text{mgs. of sugar per 100 c.c. of blood.}$$

The above method is simpler than the older blood sugar methods. The blue color of the standard is a great advantage over the older methods, where the matching of yellow colors is required.

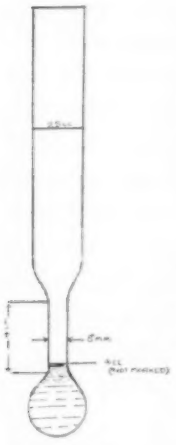

Fig. 14.
Folin sugar tube.

Sugar Tolerance.—In addition to the determination of sugar on one specimen of blood, the sugar tolerance test may be employed for the detection of diabetes and of endocrine disturbances. The sugar tolerance test consists of the determination of the blood sugar at repeated intervals, namely, before the ingestion of sugar, ½ hour, 1 hour, 2 hours, 3 hours and 4 hours after the ingestion of sugar.

One and one-half gm. of glucose is given to each kilogram of body weight. The sugar is given in water or in lemon juice on an empty stomach. In normal individuals, the blood sugar rises from normal, (80 to 120 mg. per 100 c.c.) to 130-180 mg. one hour after the ingestion of the sugar, and returns to normal within 2 or 2½ hours. No sugar appears in the urine.

In diabetes the blood sugar rises to 300 mg. or even higher in ½ to 2 hours and it takes several hours before it returns to normal.

In endocrine disturbances, the rise in the sugar curve is even slower than normal, the patient having an increased tolerance for sugar.

Determination of Nonprotein Nitrogen.—Five c.c. of blood filtrate is introduced into a pyrex digestion tube, which is calibrated at 25 c.c. and 50 c.c., and 1 c.c. of acid digestion mixture[4] is added. A glass bead is put in to prevent bumping. The mixture is now heated rapidly over a microburner until

[2] *Standard Sugar Solution.* A stock solution is made by dissolving 1 gm. of pure glucose in 100 c.c. of benzoic acid solution (2.5 gm. benzoic acid in 1 liter of boiling water, allowed to cool). Before using, it is diluted with 0.3 per cent benzoic acid one to one hundred. For each blood determination 1 c.c. of the stock solution is introduced into a 100 c.c. volumetric flask, and benzoic acid is added to the mark. Two c.c. of this standard, containing 0.1 mg. of glucose per cubic centimeter, is used.

[3] *Phosphomolybdic Acid Solution.* Thirty-five grams of molybdic acid and 5 grams of sodium tungstate are placed in a liter beaker, 200 c.c. of 10 per cent sodium hydroxide and 200 c.c. of water are added. This is boiled vigorously for twenty to forty minutes to remove nearly all of the ammonia present in the molybdic acid, cooled, diluted to about 350 c.c., 125 of 85 per cent phosphoric acid added and diluted to 500 c.c.

[4] *Acid Digestion Mixture.* Three hundred c.c. of 85 per cent phosphoric acid is mixed with 100 c.c. of concentrated sulphuric acid (free from ammonia). This is transferred to a tall cylinder, covered well to prevent absorption ammonia, and set aside for at least a week to permit sedimentation of calcium sulphate. To 100 c.c. of the clear acid, 100 c.c. of water and 10 c.c. of 6 per cent copper sulphate solution is added.

the water is boiled off. When white fumes appear the tube is covered with a small watch crystal and heated slowly at the boiling point until the solution assumes a pale green color, or at least until it fumes two minutes. The contents are allowed to cool for 60 to 90 seconds and water is then added, drop by drop at first and then to about 30 c.c. While cooling, the standard should be prepared as follows:

Into a 100 c.c. volumetric flask is introduced 3 c.c. of an ammonium sulphate solution containing 0.3 mg. of nitrogen[5], 2 c.c. of the acid digestion mixture, and water to about 50 c.c.

Fifteen c.c. of Nessler's solution[6] are now added slowly, with constant shaking, to the unknown and 30 c.c. to the standard. The blood filtrate and standard should be nesslerized about the same time. Distilled water is added to the mark and the contents of the flasks mixed thoroughly.

If the blood filtrate solution is cloudy, due to the suspensions of particles of silica from the glass, it should be centrifuged. The solution is now read in the colorimeter against the standard solution of ammonium sulphate setting the standard at 20.

The nitrogen content is calculated from the formula:

$$\frac{20 \times 30}{\text{Reading}} = \text{mg. of nitrogen in 100 c.c. of blood.}$$

Determination Urea Nitrogen.—Into a pyrex nitrogen tube are introduced 5 c.c. of the blood filtrate, two drops of pyrophosphate buffer mixture[7] and 1 c.c. of urease solution.[8] The tube is placed in water at 40° to 55° C. and allowed to stand for 5 minutes or left at room temperature for 15 minutes. Two c.c. of saturated borax solution are then added to render the solution alkaline. Five drops of paraffin oil and a glass bead are also added to prevent foaming. The mixture is distilled into a pyrex test tube graduated at 25 c.c. containing 2 c.c. of twentieth normal hydrochloric acid, the distillation consuming 5 minutes. The distillate is cooled under the tap. A standard solution is made up by placing in a 100 c.c. volumetric flask, 3 c.c. of the standard ammonium sulphate solution and about 60 c.c. of water. Standard and unknown are then nesslerized, the standard receiving 10 c.c. and the unknown 2.5 c.c. The standard is then made up to the 100 c.c. mark and the unknown to the 25 c.c. mark.

---

[5]*Standard Solution* of ammonium sulphate. Specially purified ammonium sulphate, 0.4716 g., is dissolved in 1000 c.c. of distilled water. Ten c.c. of this solution contains 1 mg. of nitrogen. For blood standard 3 c.c. is used. Toluol is added as a preservative.

[6]*Nessler's Solution.* One hundred gm. of mercuric iodide and 70 gm. of potassium iodide are introduced into a liter volumetric flask and 400 c.c. of water added. One hundred gm. of sodium hydroxide dissolved in 500 c.c. of water and cooled thoroughly is now added to the mixture in the flask, and water is added to make up a liter. The small amount of red precipitate should be allowed to settle at the bottom and the supernatant fluid should be used.

[7]*Buffer mixture.* Seventeen and two-tenths gm. of monosodium phosphate and 44.8 gm. of crystallized disodium phosphate are dissolved in 200 c.c. of warm distilled water. The solution is cooled and diluted to 250 c.c. One or two drops of Toluol is added as a preservative.

[8]*Urease Solution.* About 3 gm. of permutit powder are washed with 2 per cent acetic acid, then twice with water. Five grams of Jack bean meal and 100 c.c. of 15 per cent alcohol (16 c.c. of ordinary alcohol and 84 c.c. H_2O) are added. The flask is stoppered and shaken gently but continuously for 15 minutes after which it is filtered through a large dry filter. It should be kept in a cold place. The solution then keeps about a week at room temperature and four to six weeks in an ice box.

Urea nitrogen is calculated from the formula:

$$\frac{St \times 15}{R} = \text{mg. per 100 c.c. blood.}$$

DETERMINATION CREATININE.—Ten c.c. of the protein-free blood filtrate are measured into a large test tube. Into another tube are introduced 5 c.c. of a standard creatinine solution[9] (containing 0.03 mg. of creatinine), and 15 c.c. of water. A fresh alkaline picrate solution is prepared by mixing 15 c.c. of saturated freshly recrystallized picric acid and 3 c.c. of 10 per cent sodium hydroxide purified by alcohol. This solution is then added to standard and unknown, 10 c.c. to the former and 5 c.c. to the latter. Both are allowed to stand for 10 minutes before reading in the colorimeter, the standard being set at 20.

Calculate from the formula:

$$\frac{20 \times 1.5}{\text{Reading}} = \text{mg. per 100 c.c. blood.}$$

DETERMINATION OF URIC ACID.—Into a test tube graduated at 25 c.c. is measured 5 c.c. of blood filtrate. Into another such tube is measured 5 c.c. of standard uric acid solution containing 0.02 mg. of uric acid.[10] To each is added 2 c.c. of water, 2 to 3 drops of 20 per cent lithium sulphate solution[11] and 2 c.c. of sodium cyanide solution.[12] One c.c. of uric acid reagent[13] is added to each, the solutions are mixed and allowed to stand for two minutes. They are then placed in boiling water for 80 seconds, cooled in running water and made up to the mark with water, mixed and read against the standard which is set at 20.

$$\frac{20 \times 4}{\text{Reading}} = \text{mg. of uric acid per 100 c.c. of blood.}$$

DETERMINATION OF CHLORIDES.—Into a small Erlenmeyer flask is measured 0.115 c.c. of blood plasma and 1.6 c.c. of N/100 silver nitrate solution.[14] To

[9] *Creatinine Standard Solution.* A stock solution containing 1 mg. of creatinine per c.c. (used also for determination of creatinine in urine) is made up by dissolving 1.61 gm. of creatinine zinc chloride in 1 liter of N/10 HCl acid. Six c.c. of this solution are measured into a liter volumetric flask, 10 c.c. of normal HCl added and water to the mark. Toluene is used as a preservative.

[10] *Standard Uric Acid Solution.* A stock solution is made up as follows: Exactly one gram of uric acid is transferred to a funnel on a 300 c.c. flask. From 0.45 to 0.5 gm. of lithium carbonate is placed in a 300 c.c. beaker, 150 c.c. of water is added, and the mixture heated to 60° C. with constant stirring to dissolve the salt. The uric acid is then rinsed into the flask and with shaking dissolves practically at once. As soon as a clear solution is obtained, it is cooled under running water with shaking, and transferred to a liter volumetric flask, rinsed and diluted to 400 to 500 c.c. Twenty-five c.c. of 40 per cent formaldehyde are added and after shaking, 3 c.c. of glacial acetic acid. The solution is shaken to remove most of the carbonic acid, diluted to volume and mixed. It should be tightly stoppered and kept in the dark.

For use it is diluted 1:250 as follows: 1 c.c. is transferred to a 250 c.c. volumetric flask half full with water. Ten c.c. of 2/3 NH_2 SO_4 and 1 c.c. of 40 per cent formaldehyde are added, diluted to the mark and mixed.

[11] *Lithium sulphate solution.* Twenty gm. of powdered lithium sulphate are dissolved in about 80 c.c. of cold water. It is diluted to 100 c.c. and filtered.

[12] *Sodium cyanide solution* (approximately 15 per cent in N/10 NaOH). One hundred to 150 gm. of cyanide are transferred to a large beaker and 6.7 c.c. of N/10 NaOH is added for each gram taken, stirring occasionally until dissolved.

[13] *Uric acid reagent.* (a) Fifty c.c. of 85 per cent phosphoric acid and 160 c.c. of water are transferred to a 500 c.c. Florence flask. This is heated nearly to boiling and then 100 gm. of sodium tungstate is added. This is boiled gently but continuously for one hour over a microburner using a 200 c.c. flask filled with cold water on a funnel as a condenser.

(b) In a liter beaker is placed 25 gm. of lithium carbonate. Fifty c.c. of phosphoric acid and 200 c.c. of water are added carefully. The carbon dioxide gas is boiled off and the mixture is cooled. Solutions (a) and (b) are mixed and diluted to 1 liter.

[14] *N/100 silver nitrate solution.* 1.699 gm. per liter.

this is added 10 drops of chloride-free concentrated nitric acid. The mixture is then warmed carefully and saturated potassium permanganate solution is added drop by drop until the color it produces remains. The solution is boiled carefully for five minutes and then dextrose is added drop by drop until the color disappears. When cool, the silver chloride should lie on the bottom in little lumps and the supernatant liquid should be colorless. The excess silver nitrate is then titrated with N/100 ammonium thiocyanate solution[15] four to five drops of saturated ferric ammonium sulphate solution being used as indicator.

Chlorides are calculated from the formula:

$$\frac{\text{c.c. of silver nitrate} - \text{c.c. of thiocyanate used}}{\text{volume of blood used (0.115 c.c.)}} \times 0.0585 =$$

gm. of NaCl per 100 c.c. of blood.

Determination of Cholesterol.—With constant shaking 3 c.c. of blood are run slowly into a 100 c.c. volumetric flask containing about 80 c.c. of redistilled alcohol and ether (3:1). The mixture is heated to boiling on a water-bath, with occasional shaking. It is then cooled under the tap, made up to 100 c.c. with the alcohol-ether mixture, and filtered through a dry filter.

Ten c.c. of this extract are measured into a small beaker and evaporated just to dryness on a water-bath. The cholesterol is extracted from the dry residue by boiling out three or four times with 2 to 3 c.c. of chloroform and decanting into a beaker. The combined extracts are evaporated to less than 5 c.c. and transferred to a glass stoppered 10 c.c. graduated cylinder. The volume is made up to 5 c.c. with chloroform.

Into another graduated cylinder are introduced 5 c.c. of a standard cholesterol solution[16] (containing 0.5 mg. of cholesterol). To both standard and unknown are added 2 c.c. of acetic anhydride and, drop by drop, 0.1 c.c. of concentrated sulphuric acid. The solutions are carefully mixed and set in the dark for 15 minutes after which they are read in the colorimeter. The standard is placed at 15.

Cholesterol is calculated from the formula:

$$\frac{25 \times 100}{\text{Reading}} = \text{mg. per 100 c.c. blood.}$$

Interpretation of Blood Chemistry.—Blood sugar in children is approximately the same as that of adults, namely, 0.08 per cent to 0.12 per cent. These figures vary with the intake of food, being higher after a meal. A marked increase in blood sugar without previous ingestion of food indicates diabetes. This is of greater significance than the reduction test in urine, as the latter might not always indicate the presence of a sugar, for as is well known, salicylates and other drugs might cause a reduction of the urine by copper sulphate. There is also the condition known as renal diabetes occur-

[15] *N/100 ammonium thiocyanate solution.* 0.761 gm. per liter.

[16] *Cholesterol Standard.* A stock solution of 0.2 gm. of cholesterol in 200 c.c. chloroform is diluted 1:10 with chloroform giving the standard solution for use.

ring in nondiabetic individuals. In this condition there is a glycosuria without any increase of blood sugar.

Nonprotein nitrogen of blood in children usually runs between 25 to 40 mg. per 100 c.c. of blood. This is increased in uremia and in acute and chronic nephritis with nitrogen retention. Schloss found a marked increase in nonprotein nitrogen in cases of intestinal intoxication.

Urea nitrogen varies in normal infants and children between 12 to 20 mg. per 100 c.c. of blood. In other words, it constitutes about 50 per cent of the nonprotein nitrogen. An increase in urea may be the first indication of nitrogen retention.

Uric acid varies between 1-4 mg. per 100 c.c. of blood. Uric acid often is the first nonprotein nitrogenous product to be retained in the blood. Creatinine has a very important prognostic significance. An increase of creatinine usually means a bad prognosis. An increase in other nonprotein nitrogen constituents may not necessarily mean a bad prognosis.

Cholesterol varies between 150 to 175 mg. per 100 c.c. of blood. It is increased in diabetes and occasionally also in nephritis.

Blood chlorides vary between 500 to 600 mg. per 100 c.c. of blood, figured as sodium chloride. They are often increased in nephritis with edema and may be decreased proportionally in the urine.

Acetone and diacetic acid are normally present only in small traces, while in uremia and diabetes they may be present in large quantities.

TABLE VI

CHEMICAL CONSTITUENTS OF BLOOD IN MG. PER 100 C.C.

Nonprotein Nitrogen	25.00– 40.00
Urea Nitrogen	12.00– 20.00
Uric Acid	1.00– 4.00
Creatinine	0.9– 2.0
Sugar	80.00–120.00
Chlorides	500.00–600.00
Cholesterol	150.00–175.00
Calcium	10.0 – 11.5
Phosphorus (Inorganic)	4.8 – 6.8

REACTION OF BLOOD.—Normally, the blood is slightly alkaline. The reaction is usually expressed in terms of pH, blood having a pH of 7.4 to 7.6 compared to distilled water which is neutral or of pH 7.0. Although the blood is only slightly alkaline so far as its reaction is concerned, it has the power of neutralizing quite a large amount of strong acid before its reaction is changed. This power of the blood is due to the presence in the blood of sodium bicarbonate, disodium hydrogen phosphate and alkaline salts of proteins, which are known as "buffer substances." They constitute the so-called alkaline reserve of the blood. In diabetes, some alimentary disturbances, and some febrile conditions, the alkaline reserve of the blood is diminished. The condition is then spoken of as acidosis, although the blood is not really acid in reaction or life would be impossible.

The degree of acidosis may be determined by the examination of either the urine for increase in ammonia, presence of acetone and diacetic acid, the

TABLE VII

CHEMICAL BLOOD CHANGES IN VARIOUS DISEASES OF CHILDHOOD

	NEPHRITIS			UREMIA	DIABETES	ALIMENTARY INTOXICATION	RICKETS	TETANY
	WITH NO NITROGEN RETENTION	WITH NITROGEN RETENTION	WITH EDEMA					
Nonprotein nitrogen	Normal	Increased	May or may not be normal depending on whether or not there is nitrogen retention	Greatly increased	Normal	May be increased	Normal	Normal
Urea	"	"	"	"	"	"	"	"
Uric acid	"	"	"	"	"	"	"	"
Creatinine	"	Increased if prognosis is grave	"	Increased if prognosis is grave	"	"	"	"
Sugar	"	Normal	Normal	Normal	Increased	"	"	"
Chlorides	"	"	May be increased	"	Normal	Normal	"	"
Cholesterol	"	May or may not be normal	May or may not be normal	May be slightly increased	May be increased	"	"	"
Acetone	"	"		May be increased	"	"	"	"
Calcium	"	Normal	Normal	Normal	Normal	"	"	lowered
Phosphorus	"	"	"	"	"	"	lowered	Normal

alveolar air for carbon dioxide tension and above all, the blood for alkaline reserve or the H-ion concentration.

ALKALI RESERVE.—Normally the alkali reserve varies between 40 to 63 per cent of CO_2 at "O" temperature and 750 barometric pressure. The alkali reserve is expressed in terms of RpH.

Method.—Either the Van Slyke and Cullen apparatus or the Marriott colorimetric method may be used. The microapparatus of Van Slyke is more applicable to children's work than the macroapparatus, as only 0.2 c.c. is required in the former apparatus. The Marriott method is more frequently used in clinical work, because the colorimeter is portable and readily obtainable.

The Marriott method consists of dialyzing whole blood, or blood serum, against a salt-indicator solution having a pH of 7.0, and comparing the resulting color change with standard indicator tubes. A set of these standard tubes may be obtained from physicians' supply houses.

Fig. 15.—Alkali reserve tubes.

Preparation of Sacks.—Sacks of celloidin or Anthony's negative cotton, are used for dialysis. They are made as follows: One ounce of celloidin is dissolved in 500 c.c. of a mixture of equal parts of alcohol and ether. The solution is allowed to stand for a week to permit impurities to settle.

A test tube about 50 mm. long and 6 mm. in diameter is filled with the celloidin solution, and slowly poured out with a rotary motion. This leaves an even coating on the inner surface of the tube. After draining and drying for ten minutes the tube is filled with cold water, the upper edge of the celloidin sack is loosened and water poured between it and the tube, and the sack removed from the test tube. Several of these sacks may be made at one time and preserved by immersing them in water.

The salt indicator solution is made as follows: Eight gm. of chemically pure sodium chloride is dissolved in distilled water, 220 c.c. of 0.01 per cent

solution of phenolsulphonephthalein is added and the whole made up to one liter with distilled water. Jena glassware is used.

This solution should have a pH of 7.0, and if it does not prove so on comparison with the standard indicator tubes, should be titrated to this point with weak alkali or acid.

Procedure.—The celloidin sacks to be used are immersed in some of the salt-indicator solution. A few c.c. of blood are withdrawn with a needle from one of the patient's veins, care being taken that the blood does not hemolyze. The blood is centrifuged at once to separate the serum. One-half c.c. of the serum is measured into one of the celloidin sacks, and the sack suspended in a larger test tube containing 2 c.c. of a salt-indicator solution. The fluid level must be at least as high on the outside as on the inside of the sack. At the end of 7 minutes the sack is removed. The dialysate is transferred to a tube of the same diameter as the standard indicator tubes. A rapid cur-

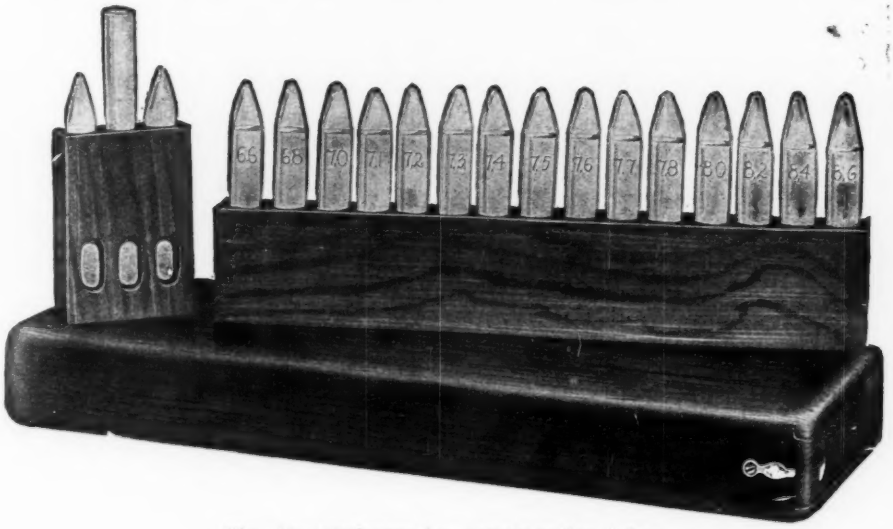

Fig. 16.—Hydrogen-ion concentration tubes.

rent of air is blown through the solution to remove the carbon dioxide. An atomizer bulb and a small glass tube is satisfactory for this purpose. After three minutes the color of the solution is compared with that of the standard tubes. The readings are given on the tubes in terms of RpH (Fig. 15). The normal values are 8.4 to 8.55 corresponding to an alveolar CO_2 tension of 35 to 45 mm. Values from 8.0 to 8.3, corresponding to an alveolar CO_2 of 28 to 35 mm. indicate moderate acidosis. Values below 7.7 indicate immediate danger of coma. The above values hold good for adults and older children. Infants give lower readings. They may show an RpH of 8.3 and yet have no evidence of acidosis.

The hydrogen-ion concentration outfit (Fig. 16) is sometimes used in clinical work to determine an advanced state of acidosis.

The following table from Palmer and Van Slyke, may be utilized clinically in the treatment of acidosis:

TABLE VIII

WEIGHT OF INDIVIDUAL		SODIUM BICARBONATE NECESSARY TO RAISE PLASMA CO_2 1 VOLUME PER CENT
kg.	lb.	gm.
19	42	0.5
38	84	1.0
57	126	1.5
76	168	2.0
95	210	2.5

ALVEOLAR AIR TENSION.—Normally the alveolar air tension varies between 40 and 70 millimeters of mercury. In acidosis less CO_2 is expired by the lungs and the alveolar air is therefore lowered. The determination of alveolar air is thus one of the methods of determining the presence of acidosis.

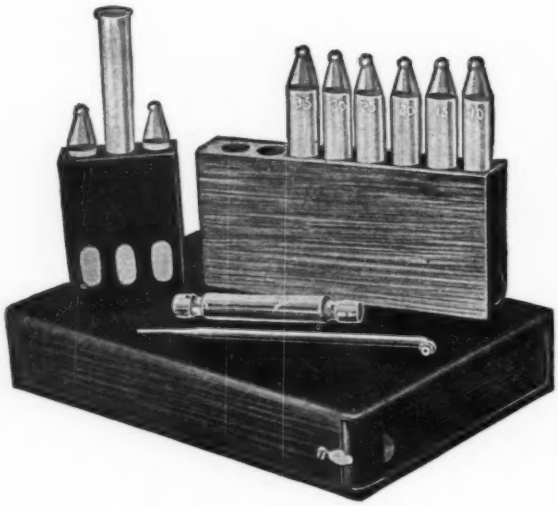

Fig. 17.—Alveolar air tubes.

Technic.—A set of standard color tubes is used. The tubes may be obtained on the market.

A standard bicarbonate solution is made either by weighing out 0.530 grams of desiccated sodium carbonate, or by measuring accurately 100 c.c. of tenth normal sodium hydroxide into a liter flask. Two hundred c.c. of 0.01 per cent phenolsuphonephthalein are added and the whole made up to the mark with distilled water. The flask containing the solution should be kept corked and paraffined.

A rubber bag of about 1500 c.c. capacity is used to collect the alveolar air. A basket ball bladder answers the purpose. For infants a bag of 500 c.c. capacity and a small rubber funnel to take the place of a mask are used. The bag is half filled with air with an atomizer bulb, and the patient breathes back and forth into the bag, 4 times in 20 seconds. The bag is then closed by a pinchcock.

Two or three c.c. of the standard bicarbonate solution are now placed

in a small test tube similar to those containing the standard color solutions. Air from the bag is then blown through the solution by a glass tube drawn out to a small bore, until the solution is saturated. When no further color change takes place the saturation is complete. The tube is stoppered and the color at once compared with the standard color tubes. The numbers on the tubes (Fig. 17) give the carbon dioxide tension, in mm. The alkali reserve of the blood may be obtained by multiplying the alveolar air tension by 0.7.

BLOOD TRANSFUSION.—

Indications.—1. Loss of blood, such as in hemorrhage of the newly born, or trauma.

2. Inanition, such as in prolonged alimentary disturbances.
3. Severe sepsis.
4. Jaundice.
5. Various anemias of childhood.
6. Hemophilia.

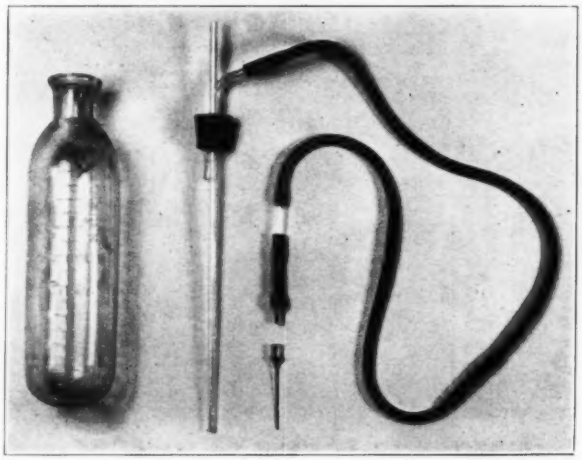

Fig. 18.—Bottle, rubber tubing, metal tip and needle (detached).

Implements Necessary.—The number of vessels and instruments necessary depends on the method to be used for the introduction of the blood into the child. If the blood is to be given subcutaneously, only 2 needles, 18 to 20 gauge, 3 Luer syringes and a tourniquet are necessary. If the blood is to be given intravenously or into the sinus the following vessels and solutions are necessary:

(a) One venipuncture needle, for the removal of blood from the donor.

(b) One needle for the introduction of blood into the child. The size of the needle depends on the route to be used for the introduction of the blood. If it is to be introduced into a vein on the child's arm or leg, or into the external jugular vein, a regular venipuncture needle is to be used. If it is to be introduced into the longitudinal sinus, a short sinus needle is to be used.

(c) Two Luer syringes of 20 c.c. capacity, to be used for the removal of the blood.

(d) Two per cent sterile solution of sodium citrate. Ten c.c. of citrate to be used for each 100 c.c. of blood.

(e) One or 2 medicine glasses as containers of the citrate.

(f) One wide mouth flask or jar into which the citrate and the blood is to be emptied.

(g) One funnel containing several layers of gauze through which the citrated blood is to be filtered from the original flask into the syphon bottle.

(h) One nursing bottle into which the blood is filtered and from which the blood is introduced into the patient's circulation.

This bottle is to be fitted with a two hole rubber stopper into which is introduced 1 straight glass tube and 1 bent tube, the latter being connected

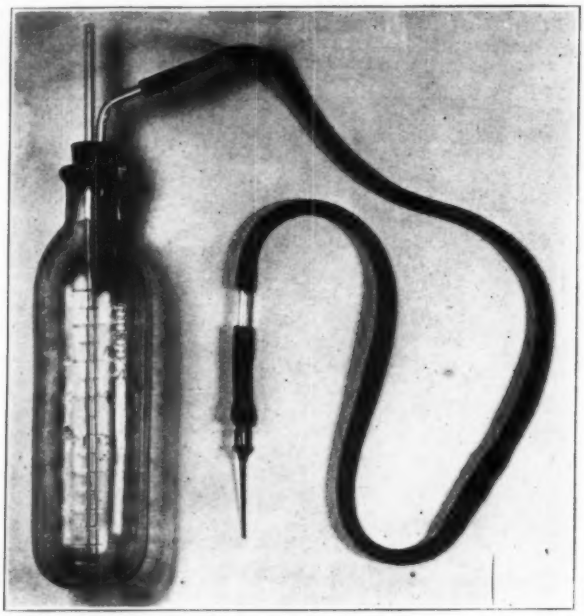

Fig. 19.—Bottle, rubber tubing and needle connected.

with rubber tubing 2 to 3 feet in length, to the end of which is attached a metal tip (Fig. 18). A clamp should be attached to the rubber tubing.

The metal tip must fit snugly into the needle by which the blood is introduced into the child. A special adapter may be used to connect the metal tip with the needle.

Technic.—A Wassermann test should be done on every donor before his blood is used. If the blood is to be introduced intramuscularly or subcutaneously, no blood grouping or matching is necessary nor is sodium citrate necessary to prevent the blood from coagulation. In that case, blood is removed from the donor's vein by venipuncture needle connected to a Luer syringe, a tourniquet being applied an inch or two above the site of the puncture. As soon as the syringe is filled with blood, it is detached from the needle, given to an assistant, who injects the blood into the child's buttocks

and meanwhile the operator fills the second syringe with blood. This is repeated until the desired amount of blood is given.

The donor is preferably seated near a table with the arm from which the blood is removed held firmly at the edge of the table.

Needless to say that every precaution is to be taken to keep the field of operation and the blood sterile.

If the blood is to be introduced directly into the child's circulatory system, the blood of the donor and the child should be grouped or matched before the transfusion is undertaken. (Technic of blood matching described in article 2). If the child is below one month of age, no grouping or matching is necessary.

The blood removed from the donor by venipuncture is introduced into the flask containing the sodium citrate. The blood is then filtered through gauze into the nursing bottle.

Another needle is now introduced into the vein or into the longitudinal sinus of the child. The clamp over the rubber tubing is loosened and the blood allowed to run to the tip in order to expel air bubbles. (Fig. 19.) The blood is now run in from the inverted nursing bottle into the child's vein or sinus by means of gravity. On completion the site of puncture is sealed by collodion.

(To be continued.)

LABORATORY METHODS

A SIMPLE METHOD FOR COMPUTATION OF METABOLIC DETERMINATIONS BY THE USE OF THE SLIDE RULE*

By M. W. Hollingsworth, M.D., Anaheim, Calif.

THE long fraction needed in the computation of the basal metabolic rate has turned the attention of several writers toward some simplification of its solution. Recently, Newcomber arranged the necessary data in the form of several condensed tables and graphic curves. Some of these are constructed to read directly in logarithms, which facilitates the finding of these figures. However, any method requiring the use of the logarithms as such necessarily calls for the writing down of all the figures, adding them up, and the conversion of this sum which is also a logarithm, into the answer. J. H. Smith and M. C. Smith devised a very ingenious graph by which one arrives at the basal rate. This graph, however, makes arbitrary the use of one standard, but is accurate to within ± 1 per cent. McCaskey has simplified the calculation somewhat by using tables and a hypothetical constant. He has made optional the use of logarithms but the calculations in his formula are more quickly performed by using logarithms, so there is little, if any, actual saving of time or labor.

One year ago I instituted the use of the circular slide rule† in the laboratory of this clinic for all mathematical problems, including the determination of the basal metabolic rate. We are able to reduce the data concerned in the determination of the basal metabolic rate to the answer in less than one minute, without jotting down any intermediate data, or any logarithm whatever. Occasional checking up by logarithms has shown absolute agreement in the computations. The use of the slide rule does not limit one to any particular normal standard; one may use the twenty-four hour output of calories or the oxygen consumption per minute with the same degree of accuracy. The circular slide rule has the advantage over the straight one in being applicable to the solution of any fraction, no matter how long, without setting down any intermediate answer to use as a new starting point. One never runs off the scale of the circular instrument as it carries the problem along continuously to the end.

There are two types of the circular slide rule on the market. One bears close resemblance to the straight pattern in having two concentric discs

*Received for publication, February 13, 1923.

†I realize I am taking some liberty with the English language in speaking of a circular rule. I do this because the circular instruments follow the same principle as the straight ones.

comparable to the two rules of the straight slide rule, which can be either rotated independently of each other, or in unison. There is also pivoted to the center a transparent sector carrying a hair line, which is the counterpart of the slide on the straight type, and which (like the slide on the straight rule, this transparent sector) can be moved independently of either disc. It also has the great advantage of having a convenient thumb nut by which the sector can be quickly clamped to the outer dial for making calculations requiring a common divisor or multiplier. The concentric discs are graduated in the same manner as the gradations on the rule and the co-ordinated rule of the straight variety. This circular instrument particularly simplifies problems for the beginner as the sector is lettered opposite the places to set the numbers, thus obviating any necessity of memorizing directions. The sector is lettered "N" opposite the place on the hair line under which the multiplicand or dividend is placed ("N" literally representing the number one is working on), and "M" and "D" opposite the places in each instance where the multiplier or divisor is placed. A conspicuous arrow, marked "Answer," points to the answer on the outer dial, while a smaller arrow on the outer dial marked "Proof," gives a check answer to be read on the inner dial. For practical purposes one neglects the proof answer entirely. This type of computer is practically foolproof. Fractions such as

$$\frac{2160 \times .923 \times 4.825 \times 1440}{10.03 \times 1000 \times 1270}$$

where one usually resorts to logarithms may be reduced by simply manipulating the sector and the discs alternately, the final answer being obtained without requiring the consideration of intermediate answers.

The other type of circular computer has only one disc, but it has two transparent arms or sectors. These are pivoted to the center, the one superimposed over the other like the hands of a clock. The construction of the pivot is such that the short arm may be rotated independently of the longer without disturbing the relation of the latter to the disc. However, rotation of the long arm automatically carries with it the short one, causing both to move as one. There is no lettering on this computer to assist one. The principle upon which this rule is based necessitates the rotation of one of the arms from the original setting in turning to the answer of all simple problems of division or multiplication. If there is any doubt one cannot simply look around the scale to verify the original setting as with the double disc, but must move the arms away from the answer back to the setting. For reckoning continuous fractions the single disc type is just as convenient as the other since we do not read our answers until the end.

There is nothing formidable about the use of the slide rule. The application of the circular type to medical problems saves so much time that I will illustrate the use of both instruments described above in the calculation of the basal metabolic rate from the findings of an instrument of the portable Benedict type; it works equally well with any other type of apparatus.

We usually compute the rate for the previous ten minute period as each consecutive period is being run, and determine the last as the patient is dressing.

We will suppose that in 10 minutes, 1⅕ seconds, the patient consumes 2160 mils of oxygen with the barometer standing at 754 mm. of mercury and with the temperature of the gasometer at 20° C. We will assume a theoretical rate of 1270 calories per 24 hours. Our problem is:

$$\frac{2160 \times .924 \times 4.825 \times 1440}{10' \; 1\frac{1}{5}'' \times 1000 \times 1270}$$

We reduce 1⅕ seconds to its decimal equivalent in minutes by placing the hair line of the sector over 18 (1⅕ = 1.8) and turning the inner disc until 60 is under the hair line opposite "D." The answer points to 3 which is the answer after placing the decimal, becoming .03 minutes (one places the decimal either by simple inspection or by following certain directions that accompany the instrument; very little practice enables one to correctly point off the decimal from inspection). This simplifies our fraction which now stands:

$$\frac{2160 \times .924 \times 4.825 \times 1440}{10.03 \times 10000 \times 1270}$$

In our actual work we have this problem memorized and set down only the barometer correction as such, the duration of the test which is reduced to decimals directly from the stop watch, and the theoretical calories which all become an integral part of the final report. Taking up the solution of this continuous fraction we place the hair line of the sector over 216 opposite "M," move the inner dial until 924 is under the hair line opposite "M," then hold the two dials together and move the sector till the hair line is over 1000 on the outer dial, then turn the inner dial until 144 is under the hair line opposite "N" of the sector. At this point, the 24 hour calories produced by the patient may be read on the outer scale where the arrow points. This is unnecessary however in figuring the result. I merely mention it as we usually write this data on the report. Next hold the two dials together and move the sector until the hair line is over 127 on the inner dial opposite "M," the answer is now read under the hair line on the outer dial which is in this case 1087 or 108.7 per cent, or, as is usually given, plus 8.7. The actual working out of the test consumes less than a minute which is less time than it would take to write down the logarithms.

We will now undertake the solution of the problem with the other rule. This rule has the two arms I spoke of, one being a little longer than the other; moving the longer arm carries with it the shorter, or the shorter may be moved alone. Both arms have hair lines. Taking first the duration 10′ 1⅕″ place the hair line of the long arm (which we will designate as "L") over 60 and the hair line of the shorter arm (which we shall designate as "S") over 1.8 then move "L" until its hair line is over 10, this carries

with it "S" the hair line of which comes to rest over 3 which is the answer after pointing off the decimal.

Applying this rule to the solution of our long fraction, turn "L" until its hair line is over 216, turn "S" until its hairline is over 1003, then turn "L" until the hairline of "S" (turning "L" turns "S" with it as I mentioned above) is over 924, then move "S" until its hair line is over 10, turn "L" until the hair line of "S" is over 4825, then turn "S" until its hairline is over 127, now turn "L" until the hair line of "S" is over 144, and under the hair line of "L" we find the answer, 1088 which on being reduced to decimals (or deducting the 100 per cent) gives us our usual method of expressing the basal metabolic rate as plus 8.8.

We will now check up this problem by using logarithms. Taking the logarithms of the numerators we find them to be as follows:

2160	3.33445
.924	1.96567
4.825	0.68350
1.440	3.15836
sum	7.14198

Logarithms of the denominators:

10.03	1.00130
1000	3.
1720	3.10380
sum	7.10510

Subtracting 7.14198
7.10610

.03688 equals logarithms of 1.086 or + 8.6

This confirms the answer we obtained by the use of the slide rule. It requires but a very little practice in the use of the rule to make it indispensable to anyone dealing with mathematical problems requiring division or multiplication. Almost anyone can learn to use it in 20 minutes. For working out graphs it saves hours of labor. The first rule mentioned comes with an attachment or clamp by which it can be fastened to the ledge of a desk and operated entirely by one hand.

REFERENCES

Newcomber, H. S.: Arch. Int. Med., June 15, 1922, xxix, 748-762.
Smith, James H., and Smith, Marsden C.: Boston Med. and Surg. Jour., May 11, 1922, clxxxvi, 641-642.
McCaskey, G. W.: Jour. Am. Med. Assn., April 9, 1921, lxxvi, 978-982.

NEW ANIMAL HOLDERS AND MOUTH GAGS*

By Joseph Lepak, Chicago, Ill.

LABORATORY workers have constantly been devising simpler methods for their experimental work, and especially is this true with animal work. Heretofore it has required two or possibly more attendants to perform an intravenous injection in a guinea pig; or gastric feedings in dogs or rabbits by means of a stomach tube have been a trying, and, in fact, a troublesome procedure. During the course of recent experimental work in this laboratory it became evident that our instrumental equipment for these experiments was inefficient. It is therefore the purpose of this report to describe three

Fig. 1.—Diagram of mouth gag for dog. The horizontal bars are covered with rubber tubing.

Fig. 1A.—Mouth gag in position, showing upper and lower horizontal bars behind canine teeth. The most circular shaped bars are placed at the sides of the dog's face.

instruments which we have devised, and used with very satisfactory results for the past 18 months. These instruments are not only simple in their construction, but they also do away with the excess help that was previously required.

CONSTRUCTION OF MOUTH GAG

This instrument is of two sizes; the larger being used for the dog (Fig. 1), the smaller for the rabbit (Fig. 2). The dog mouth gag is made of iron rod about ⅛ inch in diameter. It is curved to an almost semicircular shape to fit the sides of the dog's mouth (Fig. 1*A*), and as the rod is brought forward, the two front ends are attached to their corresponding sides in a horizontal manner, the left upper to the right upper and the left lower to the right lower. These horizontal bars are parallel with each other and cov-

*From the Department of Pathology and Bacteriology, and the Laboratory of Physiological Chemistry, College of Medicine, University of Illinois, Chicago, Ill.

ered with rubber, so as to prevent any injury to the dog's teeth or lips. This instrument can be constructed by almost any laboratory worker, the size depending upon one's own individual judgment.

The rabbit's mouth gag is similar in a way to that of the dog's mouth gag, but instead of having the curved sides there are only the two parallel horizontal bars, connected by a V-shaped support (Fig. 2*A*). The rabbit's mouth gag is made of a heavy wire rod, about $\frac{1}{16}$ inch in diameter.

These instruments constructed on a smaller scale can be used as mouth gags for guinea pigs, mice, or cats.

The Use of the Mouth Gag.—The animal, dog, or rabbit, is placed between the legs of the attendant, and putting the lower horizonal bar of the gag in the animal's mouth and forcing the lower jaw downward, the instrument slips just behind the canine teeth of the maxilla; the upper bar of the gag is slipped into the animal's mouth and the upper teeth are supported on the

Fig. 2.—Diagram of rabbit's mouth gag.

Fig. 2A.—Mouth gag in position.

upper bar. In this way the animal's mouth is kept open without any injury to the soft tissues or teeth. The same attendant holding the dog can pass a stomach tube or perform any other work in the animal's mouth. This method is very simple and does away with the extra help that was previously required.

GUINEA PIG HOLDER

This instrument consists of an ordinary hemostat to which is attached (by means of soldering) a wire rod. These rods extend forward and cross each other in the form of an X (Fig. 3). A semicircular, solid, shaped piece of tin about $1\frac{1}{2}$ inches wide and 2 inches long is soldered to the union of the hemostat and wire. The winged-shaped tin is attached perpendicular to the hemostat.

The Use of the Guinea Pig Holder.—The animal is tied down to the board in the usual manner. The guinea pig holder is put around the animal's head (Fig. 3*A*) and this brings the field to be operated upon forward. This method

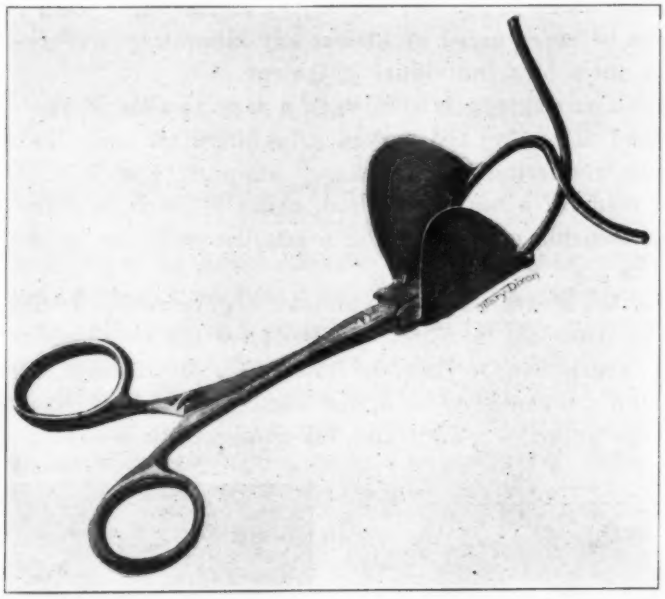

Fig. 3.—Diagram of guinea pig holder. The wires cross each other to form an X. The semi-circular tin shaped wings are attached to the junction of the hemostat and wires.

Fig. 3A.—Guinea pig holder in position showing the wing-shaped tin on each side of the guinea pig's head. The X shaped portion is behind the guinea pig's head, thus elevating the neck and bringing the field to be worked on forward. The hemostat is locked and there is no pressure on the animal's head or neck.

does away with an extra attendant holding the guinea pig's head, and also prevents the animal from biting any one working with it. We have used this holder particularly in the use of intravenous injections.

SUMMARY

Three instruments are described, one a dog and a rabbit mouth gag; the other a guinea pig holder.

These instruments are very simple to make and are great aids in saving time, simplifying the work, and decreasing the number of attendants that are ordinarily required in feeding animals by stomach tube, or intravenous injections in guinea pigs.

TECHNIC FOR THE REMOVAL AND TRANSPLANTATION OF RAT AND MOUSE TUMORS*

By Donald C. A. Butts, Philadelphia, Pa.

A TECHNIC which yields a high percentage of successful transplantations with comparative ease, and is sufficiently rapid to prevent probable loss of identity of tumor type is a factor of utmost importance. Especially is this necessary in the smaller laboratory, where the supply of tumor-bearing animals is limited and the necessity of successful inoculations is imperative.

In this laboratory we found it necessary to devise a means of tumor transplantation whereby the original animals bearing tumors might be maintained, and tumor inoculations from such animals yield a high percentage of unaltered successful "takes," in contrast to the method employed in the larger laboratories, which have an unlimited number of animals bearing the same strain of tumor at their disposal. In such cases the usual procedure is to decapitate the animal prior to removal, pathologic examination and transplantation of the tumor.

The technic employed in this laboratory is as follows:

The animal bearing the tumor (rat or mouse) is anesthetized with ether and tied, by the legs, to an animal board; the area over and directly surrounding the tumor is then carefully clipped free from hair, as shown in Fig. 1. The area is then washed with 95 per cent ethyl alcohol, painted with tincture of iodine and allowed to dry.

A smooth, rounded block is placed under the back of the animal, directly below the tumor, in order to elevate and steady the site of operation, thereby assuring greater ease to the operator.

The anesthesia, which is administered by placing the head of the animal into a cylindrical cardboard container, in which a large piece of cotton is saturated with ether, must be carefully regulated. As soon as complete general anes-

*From the Pennsylvania Department of Health Laboratories.
Received for publication, December 30, 1922.

thesia is effected the cylinder is removed and replaced with an empty cylinder. If there is any indication of the animal returning to consciousness the cylinder containing the ether should be replaced; however, signs of recovery from the ether must not be confused with those demonstrated by the administration of too much anesthetic. Generally the former condition is demonstrated by signs of pain and squealing, while, in the latter, respiration is usually very rapid, shallow and difficult, associated with quick and repeated

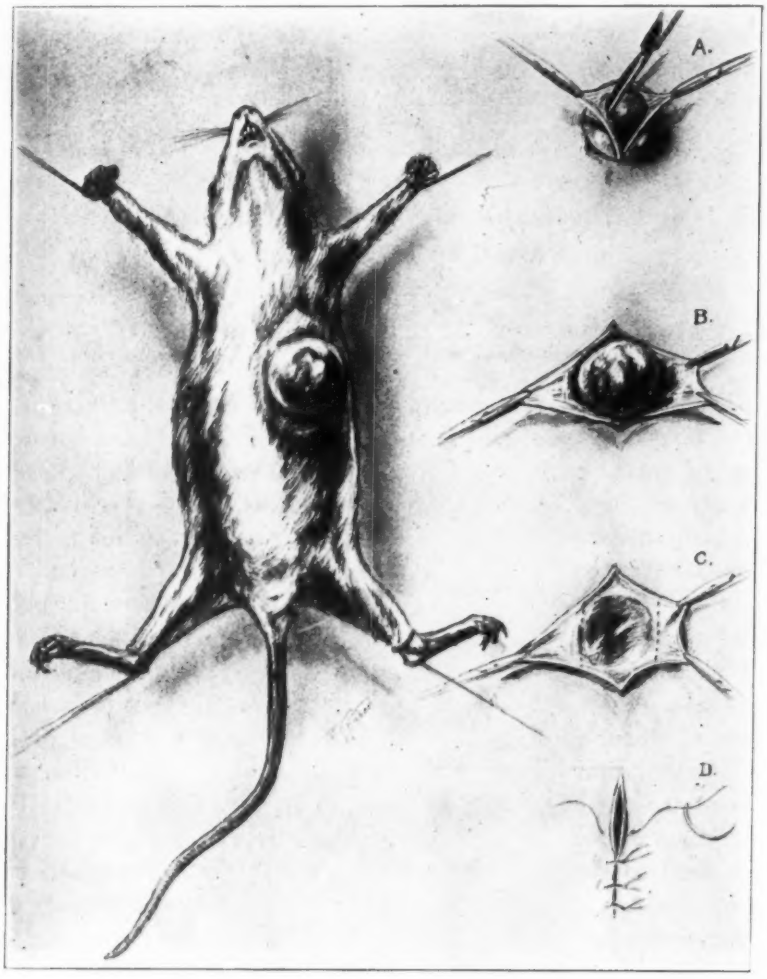

Fig. 1.

convulsive movements. If this latter condition persists after discontinuance of the ether, a small quantity (0.5 c.c.) of sterile saline solution may be injected, usually subcutaneously. Therefore proper anesthesia, with special care during the induction, is essential.

Before starting the operation all instruments and materials to be used should be sterilized in the regular manner. This is imperative, otherwise severe postoperative infections may result.

The following constitutes the necessary material to have on hand when starting the operation: 1 small scalpel; 2 dissecting forceps; 3 hemostatic forceps; 1 pair iris scissors; 1 tube of No. 4 silk; 1 tube of No. 00 catgut; 1 curved suturing needle; 1 sterile Petri dish; 1 lumbar puncture needle; 1 Luer syringe (1 c.c. capacity); flask of sterile saline solution; sterile cotton; bottle of 95 per cent ethyl alcohol; small bottle of formalin solution (4 per cent).

To perform the operation, the operator and assistant take their positions on opposite sides of the table or animal board. The incision is made directly over or at the base of the tumor. When the tumor is large with the central portion necrotic, an incision around one-half the diameter at the base of the tumor is advised; under other conditions an incision directly over the tumor is usually employed; however this point remains one of choice to the operator.

Each edge of the incision is grasped with a hemostat and carefully held by the assistant. The skin is incised until the encapsulating membrane of the tumor is reached (Fig. 1*A*). The assistant now grasps the tumor and draws it up and towards him, while the operator draws the border of the incision nearest to him down and away from the tumor. In this manner the subcutaneous tissue may be separated from the tumor on one side and by reversing this manipulation the other side of the tumor is freed from its subcutaneous connection (Fig. 1*B*). The tumor is now held between the thumb and index finger and the remaining connections severed with scissors or scalpel (Fig. 1*C*). When completely excised it is placed into a sterile Petri dish.

Slight bleeding encountered during the operation is controlled by immediate compression, whereas, profuse bleeding necessitates ligation of the bleeding points with silk thread. After all bleeding has been checked and the excess skin is clipped off (indicated by dotted line, Fig. 1*C*), the wound is closed by suturing the borders of the incision with No. 4 silk or No. 00 chromic catgut (interrupted sutures are employed), the number depending upon the size of the incision, one stitch being required for each ¼ inch (Fig. 1*D*). The line of incision is painted with tincture of iodine and the animal placed in a separate cage until entirely recovered from the anesthetic, usually about twenty minutes. No dressings are necessary following operation; food and water are permitted. The time occupied in performing the operation, including etherization and suturing, is generally about fifteen minutes.

The edges of the incision in cases where the tumor is not adherent to the skin, are healthy and heal by first intention; but in cases where the skin has become adherent and more or less necrotic, sufficient cutaneous tissue must be removed with the tumor in order to secure smooth, regular and healthy edges to permit suturing.

Inoculation of the Tumor.—The method of inoculating the animals is the one generally employed and will be outlined as follows:

The tumor is held with sterile forceps and cut through the center horizontally to the long axis with a sterile knife or scalpel. One-half is placed in the formalin solution for sectioning and pathologic examination, while the other half is used for the inoculations. A portion of the border is cut free

from necrotic matter which may be present, and cut into small pieces (about 0.03 gm., or about one-half the size of a pinhead). A lumbar puncture needle is flamed in the Bunsen flame and wiped off with a piece of cotton soaked with saline solution and then with a piece of cotton moistened with ethyl alcohol. The fragment of tumor is introduced into the bore of the needle with the point of the scalpel. The point of the needle is introduced subcutaneously at the groin and pushed up to the axilla where the piece of tumor is deposited by pushing in the plunger of the needle. If the transplantation is successful, a nodule can usually be found in the inoculated animals after fourteen days.

After using this method of excision and inoculation over a period of two years, in which time over two hundred tumors were removed and approximately one thousand animals inoculated, there were only two deaths as the result of operation, both due to poorly regulated anesthesia. In no case have the inoculated animals developed any infection and have yielded an extremely high percentage of successful transplantations.

Other advantages possessed by this technic are, that a small fragment of the tumor, or where multiple tumors exist, several may be left in the host unaltered for further observation and transplantations. Moreover, the normal increase in this colony is not inhibited, evidenced in the animal represented in Fig. 1, which gave birth to six young directly after operation, the nursing and care of which were normal in every respect.

The Journal of Laboratory and Clinical Medicine

VOL. VIII ST. LOUIS, MO., MAY, 1923 No. 8

Editor-in-Chief: VICTOR C. VAUGHAN, M.D.
Chicago

ASSOCIATE EDITORS

DENNIS E. JACKSON, M.D. - - CINCINNATI
PAUL G. WOOLLEY, M.D. - - DETROIT
FREDERICK P. GAY, M.D. - WASHINGTON, D. C.
J. J. R. MACLEOD, M.B. - - - TORONTO
ROY G. PEARCE, M.D. - - AKRON, OHIO
W. C. MACCARTY, M.D. - ROCHESTER, MINN.
GERALD B. WEBB, M.D. - COLORADO SPRINGS
WARREN T. VAUGHAN, M.D. - RICHMOND, VA.
VICTOR C. MYERS, PH.D. - - NEW YORK
MARSH PITZMAN, M.D. - - - ST. LOUIS

Contents of this Journal Copyright, 1923, by The C. V. Mosby Company—All Rights Reserved

Entered at the Post Office at St. Louis, Mo., as Second-Class Matter

EDITORIALS

Vaccine Treatment in the Presence of Infection

IN a recent series of articles Wright has ably summarized his investigations and conceptions with regard to therapeutic inoculation. Although Jenner stands as the originator of vaccination, it was Pasteur who first developed a clear understanding of the principles involved and applied them, particularly in his work on anthrax and rabies. Pasteur's conceptions may be briefly summarized somewhat as follows. In prophylactic inoculation, the pathogenic microorganism, or in case such is unknown, the virus containing the specific pathogen must be obtained. The vaccine must consist of the living germ whose virulence has been attenuated. The exact amount introduced into the animal to be immunized is not of great importance. Vaccines should be given only to uninfected animals, or if infected, only in case the incubation period still has more than ten days to run. It should be administered subcutaneously. Protection is obtained only after ten or more days from inoculation and is specific in that vaccination with one pathogen will not protect against another unrelated organism.

Following the work of Pasteur and particularly in the development of typhoid immunization, it developed that the antigen need not necessarily be

a living attenuated microorganism, but may consist of killed virus. In fact, it was soon recognized that dead virus was more active in producing immunity. The explanation appears to lie in the principle formulated by Ehrlich; *corpora non agunt nisi soluta.*

In typhoid vaccination it was found that better results were obtained when a severe reaction was avoided. The bactericidal power of the blood passes through a negative phase following administration of quantities of typhoid vaccine great enough to produce severe constitutional reaction. Following this there comes a positive phase in which the bactericidal power may be increased as much as a thousand times. With large doses the negative phase becomes protracted and may sometimes last indefinitely. Following doses which produce a minimum of constitutional disturbance, the negative phase disappears, the positive phase beginning immediately, so that often as soon as twenty-four hours after inoculation, the bactericidal power of the blood is decidedly enhanced. This fact necessitates some change in the Pasteurian code, since vaccine therapy may, theoretically at least, be given with good results when the incubation period has much less than ten days to run. This has occasionally been demonstrated to be true in inoculation during the incubation period of the plague. Presumably the immunizing process occurs in those areas of the body which are still uninfected. Furthermore, the quantum of vaccine administered, contrary to the tenets of Pasteur, is of considerable importance in determining the rapidity of rise in bactericidal power and the presence or absence of a negative phase.

Wright's early modification of the Pasteurian code contained the following essential points. We must possess the microbe or the virus of a particular disease in order to manufacture a vaccine. Stock vaccines made from the specific microorganism give results comparable to those obtained from autogenous vaccines. The vaccine should, wherever possible, be sterile. Vaccines may be used in prophylaxis, in preventive treatment during the incubation period of general infections and in localized infections not accompanied by fever and frequent autoinoculations. Bacterial vaccines should be incorporated hypodermically. Small doses of vaccine produce a positive immunizing effect within twenty-four hours. Larger inoculations, like heavy autoinoculations, result in a negative phase which is more pronounced the greater the quantity of antigen.

The dosage should be that which promotes the optimum immunizing or "epiphylactic" response. If in obtaining this optimum result, sufficiently large doses are necessary to produce a temporary negative phase, the latter is permissible in view of the end result desired. Reduced doses should be employed when the chief matter of concern is to obtain rapid clinical improvement. When the patient is infected, the dose should stand in inverse relation to the volume of his infection.

Wright also hypothecated in his earlier work that the anti-bacterial substances elaborated in response to inoculation operate specifically upon the variety of microbe which has furnished the vaccine with the possibility of some slight collateral immunization.

While this second code of Wright's differs quite radically from the Pasteurian code, his third is at even greater variance with the second. First he assumes that although the nature of the infecting microbe should be known, it is not necessary that the vaccine be derived from the species causing the disease. It is sufficient to have demonstrated that the vaccine used, no matter what its source, increases the concentration and action of those anti-bacterial substances which operate upon the specific infecting microbe. This hypothesis tends somewhat in the direction of the more recent conjectures with regard to so-called nonspecific immunity.

The second radical departure lies in the method of inoculation. On the assumption that the bactericidal substance is produced in the blood, direct introduction of the vaccine into the blood stream will call forth prompter reaction. "When vaccines in appropriate doses are added to the blood either *in vivo* or *in vitro,* instantaneous epiphylactic or immune response is evoked and the maximal response may be expected after only very short delay."

This reaction, according to Wright, consists in an extrusion of bactericidal elements from the leucocytes. To only an insignificant extent is the germicidal action due to phagocytosis. The anti-bacterial substances derived from the leucocytes are polytropic, exerting their action not only upon homologous but also upon quite unrelated species of microbes. Wright finds that normal blood is much more highly destructive to staphylococci than is blood from a case of streptococcus septicemia. In the latter blood there is an impairment of the destructive mechanism against microorganisms other than that causing the infection.

In septicemias and other heavy bacterial infections, the leucocytes lose their power of responding to vaccines. Here it is necessary to determine whether the blood still retains its power of epiphylactic or immune response. If it does, vaccination may logically be employed even in these conditions. Where there is no such response the appropriate treatment would be by immuno-transfusion, transfusion with healthy blood which has made the proper epiphylactic response.

Wright finds that in normal bloods, two bactericidal mechanisms play a part. If two specimens of the same blood be implanted with bacteria, the first with a few, the second with a large number, one would expect to find a greater proportion of bacteria destroyed in the former. As a matter of fact, the germicidal efficiency of the serum is greater with the higher implantation. The presence of large numbers of bacteria apparently calls forth some secondary immunity reaction which remains inactive when only small numbers of bacteria are present. With implantations of 1000 staphylococci per c.c. an average killing of over 75 per cent is observed, while with implantation of less than 150 staphylococci per c.c., none were killed. The suggested interpretation is that we have in the blood an apparatus for primary and one for secondary defense, termed aphylactic and epiphylactic. The former probably depends upon phagocytic action and the bactericidal action of serum and deals with small bacterial implantations. The epiphylactic response develops from anti-bacterial elements given out by the leucocytes when these are incited by

antigen. The epiphylactic response is far more efficient than the aphylactic. Blood implanted with 33 microbes per c.c. cannot kill one, but when implanted with 10,000, it can kill 2,000. This indicates that some new method of response has been evoked.

In streptococcus endocarditis where there are few microorganisms in the blood, with only moderate pyrexia, little constitutional disturbance and a long standing progressive course, the machinery of first defense has failed to perform its work and the epiphylactic machinery has never been called into action.

In those cases of bronchopneumonia which recover, Wright hypothecates that the epiphylactic machinery has been effectively although tardily brought into operation. In the fatal cases, this higher response has been put out of action by overwhelming bacterial intoxication. In septic cases, such as advanced tuberculosis and localized abscesses, the epiphylactic defense reaction is called forth whenever large amounts of bacteria are delivered into the blood stream and with excellent though temporary results.

The question naturally arises as to what is the proper vaccinating dose in a given case of infection. The proper dose would be the number of bacteria which would, together with the bacterial elements already in circulation, evoke in the patient's blood a maximum immunity response. In prophylactic inoculation, the active principle supplied from without will not be supplemented from within and we may therefore employ a considerable dose of vaccine. In infection, the volume thereof must be considered and a proportionately smaller amount of vaccine administered. When a hyper-vaccinating dose is already circulating in the blood, we should abstain from therapeutic inoculation.

The dead microbes provide the therapeutic agent but the living decide the issue. The magnitude of the response reaction will depend upon the dose of vaccine, but the effectiveness thereof depends upon the number of living microorganisms to be dealt with. There is an upper limit to the number of bacteria in the blood which can be taken care of even by the mechanism of the epiphylactic response. Laboratory experiments would indicate that the epiphylactic reaction can control infection with hundreds or perhaps thousands of bacteria per c.c., but it is not competent to control heavier infections. However, even in the severe streptococcus septicemias, the blood infection rarely exceeds several hundred per c.c. But it is more difficult to attack the millions of microorganisms not in the blood stream, but growing in body tissues.

Wright's work indicates that the killing of the bacteria in the epiphylactic reaction is practically instantaneous, fully two-thirds being destroyed in this way. That the germs are killed instantly and at ordinary temperature gives conclusive evidence that phagocytosis is not the cause of their destruction. This latter phenomenon begins only after an appreciable delay, especially at room temperature. The conclusion reached is that the action depends upon some bactericidal property of the serum. This high bactericidal power is not present normally but suddenly develops upon the implantation of large

concentrations of bacteria. The body tissues play no part in the sudden access of power, because it can occur *in vitro*. Wright believes that this increased bacterial efficiency depends upon the extrusion from the leucocytes of a nonspecific destructive poison. In support of this he draws attention to the fact that when washed leucocytes are implanted on cultures of staphylococci or streptococci, the latter are killed. This destruction occurs without phagocytosis.

This opens the way to an understanding of the possible existence of nonspecific immune reactions. The hypothetical substance which kills in the epiphylactic response will act upon all foreign bacteria. The anti-bacterial agent is polytropic. Again in favor of this is the observation that the blood kills instantly, before time has elapsed for the elaboration of a specific substance. We can more readily conceive of a general immune substance than of a specific reagent for each and every antigen. This at least, as far as elements to be derived from the leucocytes are concerned. Thus it has repeatedly been observed that an individual does not develop more than one exanthem at a time.

The suggestion is made that the leucocytes may be stimulated to the extrusion of the bactericidal substance by chemical agencies. Salvarsan in syphilis complicated with streptococcus infection may eliminate the latter. Blood for a few hours after the administration of salvarsan becomes bactericidal to streptococcus and staphylococcus. The elaboration of diphtheria antitoxin and of agglutinins to the colon bacillus may be appreciably increased by the exhibition of manganese chlorid. The editor observed in one of the camp hospitals during the influenza epidemic that those patients who had recently received salvarsan were less prone to ward infection with influenza than were those who had not been so treated.

Large doses of vaccine administered subcutaneously are fed slowly into the blood stream, and if the rapidity of absorption into the blood happens to be just right, as good results may be obtained as by the intravenous injection of the proper dose for epiphylactic response. On the other hand, if the vaccine enters the blood too rapidly a formidable negative phase may supervene. Finally, if a relatively small dose is given subcutaneously, the concentration in the blood may never reach that desired for maximum response.

Wright has developed a "vaccine response test" by which he determines *in vitro* whether a patient is or is not capable of making an immunizing response. When such response can be made, it is possible in addition to ascertain the requisite dose of vaccine which, when injected intravenously, will produce the optimum reaction. In those cases in which the response action will not occur, the transfusion of immune blood may be immediately resorted to without wasting more time on vaccine therapy.

In this procedure the first fact to be ascertained is the bactericidal efficiency of the patient's blood. This is done by comparing with the efficiency of a control blood. A micromethod is described, utilizing 50 c.mm. of blood. This test requires twenty-four hours' incubation, and in urgent cases may

be dispensed with. The second step in the determination of the immune response reaction consists of a series of laboratory tests termed the "chiastic method." Four phagocytic mixtures are made, the first containing patient's serum and washed leucocytes, the second containing normal serum and washed leucocytes, the third patient's serum and normal leucocytes, and the fourth, normal serum and the patient's corpuscles. The mixtures are made in equal proportions and to each is added a similar volume of a suitable bacterial mixture. These are incubated and read as in the familiar technic for the determination of the opsonic index. The *opsonic index* of the patient's serum is obtained by dividing the phagocytic count of preparation 3 by that of preparation 2; the *phagocytic index* of the patient's blood, by dividing the phagocytic count of preparation 1 by that of preparation 2. The *phagocytic efficiency* of the patient's leucocytes is expressed by the fraction obtained by dividing the count of preparation 4 by that of preparation 2. According to Wright, the premonitory signs of a grave infection would seem to be a reduction of the leucocytic efficiency coupled with a rise in the opsonic index and a moderate fall in the phagocytic index. In the later, graver stages of infection, the opsonic power is also reduced and the whole triad of phagocytic function gradually falls until at the extreme limit, zero is reached in each case.

In those cases where the bactericidal power of the blood has been found deficient, it remains to determine whether the patient has any capacity of making immunizing response and with what vaccine and what dose he can make the optimum response. This is done by the introduction of graduated amounts of vaccine into a series of volumes of the patient's blood and seeing whether there is any improvement in the hemobactericidal or phagocytic power of the vaccinated blood or in the serobactericidal or opsonic power of the serum. If time does not permit the culturing of the blood for determination of bactericidal power, we may utilize the measurement of the phagocytic power of blood and the opsonic power of serum to which the graduated doses of vaccine have been administered. Thus the phagocytic index of one patient's unvaccinated blood was 1.8. The same blood inseminated with twenty, forty and eighty streptococci per c.c. showed a phagocytic index of 1.6, 1.9 and 1.4 respectively. This showed no advantage over the uninoculated blood. However, the same blood vaccinated with 160 per c.c. showed an index of 3.2, a decidedly better response reaction. The conclusion was that the patient's blood still possessed a considerable capacity for immunizing response and that vaccination with 160 killed streptococci per c.c. would give the best response. The patient was therefore inoculated intravenously with 700,000 streptococci with excellent clinical results.

The three sequential points enumerated by the author in the treatment of infection are, first, the removal of localized accumulations of pus; second, intravenous administration of vaccine in those cases which are found capable of immunizing response; and third, immuno-transfusion for those cases so overwhelmed with infection that they are incapable of producing an optimal epiphylactic response reaction.

The author reports cases showing improvement in the phagocytic power or index of the patient's cells after evacuation of pus, after therapeutic inoculation and after immune transfusion. The donor for transfusion is treated by intravenous inoculation of that dosage which has been found to call forth a maximum epiphylactic reaction in his own blood. He is bled one-half hour later.

Whether or not Wright's work of the past few years, as summarized in his series of articles, becomes generally accepted, it does open several interesting avenues of thought toward which many immunologists are independently turning. These may be summarized as follows.

The results of vaccination in the presence of infection are not always due to a specific antigen-antibody reaction.

The quantum of vaccine to be administered is all important and must be determined for each case. It may be incorporated intravenously.

This quantum may be roughly determined within a short period by the measurement of a series of phagocytic reactions.

REFERENCES

Wright, Sir Almroth E.: New Principles in Therapeutic Inoculation, Lancet, London, Feb. 24, 1923, cciv, 365.
Wright, Sir Almroth E.: Ibid., March 3, 1923.
Wright, Sir Almroth E.: Ibid., March 10, 1923.

—*W. T. V.*

The Black Death of the Fourteenth Century

OCCASIONALLY a physician meets with a layman who has read Hallam, Creighton, Gasquet, or some one else who has written on the epidemics of the middle ages, and the physician is asked, what was the nature of these diseases, are such still in existence, can they be identified, how do the symptoms as reported at that time correspond with those in evidence now, and do the older descriptions contain marked exaggerations? One of the great epidemics described by the authors referred to is known as the black death of the fourteenth century. We are told that this epidemic within a few years killed 25,000,000 people in Europe, reducing the total population about one-half. The literature concerning the black death is fairly satisfactory and enables us to trace it from Bagdad, or at least from that region to Europe, and to follow it as it flowed out to the north and west. At that time caravans carried the merchandise, which had been assembled at Bagdad, from China and India, by several routes to Europe. There were, however, two of these trails which were most generally taken. One followed the Tigris from the plains of Mesopotamia northward, passed over the mountains and reached the shores of the Black Sea at or about the present city of Trebizond. The other, better known and by far the shorter route, passed westward from Mesopotamia, finally reaching the eastern end of the Mediterranean Sea. Some of the merchandise passed around through northern Egypt from whence

it was shipped across the Mediterranean. For some years the epidemic had raged in India and China and, even as now, the virus had its home, or at least a home, in the Himalayas. At that time the rival cities of Genoa and Venice were in earnest contest, sometimes with military accompaniments, for the trade of the east. Merchant ships, often armed, were making regular trips across the eastern end of the Mediterranean. In the closing months of the year 1347 infected ships reached both Genoa and Venice. Gabriele de Mussi has left a graphic description of the epidemic as it entered Genoa. He tells the story as though he was on an infected ship. He writes: "Alas! our ships enter the port, but of a thousand sailors hardly ten are spared. We reach our homes; our kindred and our neighbors come from all parts to visit us. Woe to us, for we cast at them the darts of death! Whilst we spoke to them, whilst they embraced us and kissed us, we scattered the poison from our lips. Going back to their homes, they in turn soon infected their whole families, who in three days succumbed, and were buried in one common grave. Priests and doctors visiting the sick returned from their duties ill, and soon were numbered with the dead. O, death! cruel, bitter, impious death! which thus breaks the bonds of affection and divides father and mother, brother and sister, son and wife. Lamenting our misery, we feared to fly, yet we dared not remain."

In the Decameron, Boccaccio gives even a more striking picture of the plague as it occurred in Florence. The most frequently quoted paragraph from this racy author, so far as he treats of the black death, runs as follows: "What magnificent dwellings, what stately palaces, were then rendered desolate, even to the last inhabitant! How many noble families became extinct! What riches, what vast possessions were left with no known heir to inherit them! What numbers of both sexes, in the prime and vigor of youth, who in the morning, Galen, Hippocrates, or Esculapius himself, would have declared in perfect health, after dining heartily with their friends here, have supped with their departed friends in another world."

Living amidst the plague in Parma, Petrarch wrote: "Alas! my beloved brother, what shall I say? How shall I begin? Whither shall I turn? On all sides is sorrow; everywhere is fear. I would, my brother, that I had never been born, or at least, had died before these times. How will posterity believe that there has been a time when without the lightnings of heaven or the fires of earth, without wars or other visible slaughter, not this or that part of the earth, but well-nigh the whole globe, has remained without inhabitants?"

Probably there is not in the whole range of literature a more striking paraagraph than the following written by an Irish monk: "And I, Brother John Clyn, of the Order of Minorites and the convent of Kilkenny have written these noteworthy things, which have happened in my time and which I have learned as worthy of belief. And lest notable acts should perish with time, and pass out of the memory of future generations, seeing these many ills, and that the world is placed in the midst of evils, I, as if amongst the dead, waiting till death do come, have put into writing truthfully what I have heard

and verified. And that the writing may not perish with the scribe, and the work fail with the laborer, I add parchment to continue it, if by chance any one may be left in the future and any child of Adam may escape this pestilence and continue the work thus commenced."

Other striking illustrations might be taken from the literature of the time, but we turn to inquire as to the nature of the black death. This inquiry is easily and certainly answered. Fortunately, Boccaccio was not the only chronicler of the epidemic as it ravaged Florence. There was in that city and at that time a more scientific observer. His name was Villani. This author says that the disease manifested itself in two forms. In one the lungs were evidently involved; there was coughing and spitting of blood, and from this form none recovered. In the other form, the glands in the inguinal and axillary regions were swollen, often ruptured and discharged, and from this form of the disease many recovered. At Avignon, then the seat of the papacy, there was the greatest surgeon of the time, Guy de Chauliac, physician to the pope and a man whose writings dominated surgical theory and practice for several centuries later. His testimony as to the nature of the disease is confirmatory of that furnished by Villani. Chauliac says that some patients had constant fever, accompanied by spitting of blood, and from this form of the disease all patients died in three days. Many, however, recovered from the bubonic form. Other Italian physicians repeat these descriptions so closely that there is no break in the character of the testimony. In France, Covino named the disease *pestis inguinaria*. In the remainder of Europe there appears to have been at that time no physician who recorded anything of value pertaining to the nature of the disease. The black death was without question the disease which we now recognize as the plague and it manifested itself, then as now, in both the pneumonic and bubonic form. It is worthy of remark that in the nearly 600 years which have elapsed since the prevalence of the epidemic about which we are now writing there has been no important or even recognizable change in the symptoms or lesions induced by this infection.

As has been stated, the black death found its way into Italy simultaneously by Venice and Genoa in the last month of the year 1347 or in the first month of the following year. We are somewhat surprised that it advanced so rapidly, reaching France and, indeed, spreading all over that country by the early summer and appearing at English seaports only a few weeks later. It spared neither city nor hamlet and it is interesting to make some inquiry concerning the living conditions of the people in Europe at that time. Recently, a Frenchman, M. Luce, has investigated this matter. He thinks that France at that time had a population of four or five million, possibly more. He says that there were numerous villages scattered rather thickly over the country, the most of which have long since disappeared. The huts of which these villages were composed were built of mud and clay, sometimes containing cavities filled with straw. As a rule, dwellings were of only one story. The public houses frequently had two. The roofs were thatched or covered with wood or stone. Windows were the exception and where

they did exist were mere slits in the clay walls closed with shutters. The doors were fastened with wooden latches, with open spaces above for the escape of the smoke, as in many of these huts there were no chimneys. It is more than likely, although on this point we have no direct testimony, that these huts housed more rats than human beings. We are told that before the black death appeared among the people in Florence large numbers of rats came into the streets in open daylight without apparent fear of man and that many of them died in the gutters.

Many cities or large villages in western Europe were so badly crippled by the black death that they have never fully recovered their position. Two years before this epidemic Yarmouth must have been a very flourishing English port; at least it contributed 43 ships and 1,950 sailors to Edward III who, at that time was moving on France, while at the same time London was able to supply only 25 ships and 662 mariners. Before the plague the great church at Yarmouth was not ample enough to accommodate the population. Since that time it has never been crowded. Before the black death it is said that the University of Oxford was attended by 30,000 students annually, a number never since approximated; indeed, in the year 1349 the king addressed the bishops and among other things he urged them to support the University of Oxford, which he stated was standing "like a worthless fig tree without fruit"

Do our older authors exaggerate the mortality caused by the black death? This is a question not easily answered. There were no vital statistics anywhere at that time. We can only guess at the population of England, France or Italy in the fourteenth century. Gasquet, a learned authority in church matters, has tried to figure out the mortality caused by the black death in England in this great epidemic. He concludes from a study of the monasteries and convents of that time that about half the population of England and Wales was destroyed by the black death. He thinks that before the plague the total population of England and Wales was somewhere between four and five million and he states that from the subsidy roll of 1377—some 27 years after the great mortality—the population was found to be 2,350,000. It is quite likely that the mortality among the religious orders was higher than it was among the people in general because of the crowded condition of the convents and monasteries. In England at least, the black death so thinned out the working classes that those who were left alive dictated their own wages and markedly improved their living condition. Gasquet says that in England alone about 25,000 priests were killed and that this so reduced the number of teachers in the classical languages that for the first time the vernacular was used in English schools.

—*V. C. V.*

The Standardization of Biological Stains

ON March 2nd at the Chemists Club in New York City there was held a meeting of the Executive Committee of the Commission on Standardization of Biological Stains. The members of this Committee are: H. J. Conn, Geneva, F. B. Mallory, Boston, L. W. Sharp, Ithaca, N. Y., J A. Ambler, Washington, D. C., and S. I. Kornhauser, Louisville, Ky. The meeting was also attended by C. H. Herty to represent the Synthetic Organic Chemical Manufacturers Association, and by F. P. Garvan and W. F. Keohan to represent the Chemical Foundation. The meeting is a matter of interest to everyone in the medical profession.

All physicians realize the need of dyes for staining specimens in the laboratory diagnosis and investigation of disease. It is not perhaps so generally realized that the dyes used for this purpose, in order to give constant results, must be of very precise chemical composition; and yet it is a very difficult matter for either the chemist or the biologist to control their composition. Before the war all stains were imported from a single German firm. This firm did not manufacture stains, but bought textile dyes in batches of considerable size, and after some preliminary testing bottled them and sold them under its own name to the biological laboratories of the world.

When the war broke out the American laboratory was deprived of this foreign source of stains. After the pre-war stocks already on hand had given out, much difficulty was experienced in getting stains of the quality necessary. The Society of American Bacteriologists began an investigation of American-made dyes that were being sold as biological stains. The results of this investigation were so promising that it proved possible to secure the assistance of the National Research Council through whose agency a cooperative investigation was arranged among the members of several national societies. Recently the work has been organized under a special commission independent of the Research Council but still representing the different national societies that were cooperating in the earlier work.

At the executive committee meeting of this Commission just held, the very encouraging results of the work were reported. It was shown that already the stains available in America are in practically all cases as good and sometimes better than the best of the pre-war stains. The most important fact brought out at this meeting was that while the pre-war stains were standardized only in an empirical way, by buying large batches without knowing the exact composition of the dye, they must now be standardized on the basis of pure chemicals.

The reason for this is because it is proving that in some cases the impurities present in the pre-war stains were very necessary. Sometimes these impurities were other dyes and sometimes supposedly inert materials like dextrin. In all such cases the task plainly before the Commission is to find out what the impurity is which was responsible for the good staining qualities

of the impure product. Then in the future the users of stains must demand that these impurities be present, not as impurities, but as intentionally added ingredients. When this has been done and the products are labeled and used accordingly, the American stains will become standardized in a true sense of the term.

Very shortly the Commission will begin issuing certification of definite batches of stain that it has found satisfactory. These stains will be put on the market under a special label bearing the name of the Commission. Users of stains must be on the lookout for products bearing this label. Buyers of stains must also watch for spurious imitations of this label put out by unreliable concerns. Any form of certification appearing on a stain label not bearing the name of the Commission is merely a certification by the manufacturer or dealer himself, and as such has no value.

The Chemical Foundation has very kindly agreed to support the work of the Commission financially.

—H. J. Conn, Chairman, Executive Commission.

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, 404 Professional Building, Richmond, Va.)

*Clinical Medicine**

IN this volume, the author comes into closer contact with his readers through the easy style of classroom conversation. It is true that in undertaking this method of presentation, one cannot go into exhaustive detail in the discussion of each subject. A critic might therefore complain of superficiality, but on the other hand, the important features and the newest facts are driven home in a manner that cannot be easily accomplished in more formal rhetoric.

In connection with the first case reported, the author applies his theories of the rationale of a diagnostic survey. He has discussed this subject often in the literature of the past few years, but in no place is it as concise and readily followed as in this volume.

The cases selected cover the various types of disease, so that the book as a whole, with its discussion of differential diagnosis, etc., forms an excellent teammate for a general treatise on internal medicine.

It is the type of book that one may pick up of an evening and read for pleasure.

Thurston's Pharmaceutical and Food Analysis†

ASIDE from the fact that the title of this book may be a little misleading, it is an extremely valuable volume. The first portion of the book deals with general methods of analysis and discusses in detail the polariscope, refractometers, specific gravity apparatus and other methods of analysis of fats, oils and waxes, such as the determination of acid value, boiling point, iodine value, saponification, etc. Following this is a chapter devoted to oils, fats and waxes in which the source, principal components, preparation, properties, uses and byproducts of each is thoroughly discussed. There follow chapters on dairy products, methods of analysis and detection of adulteration, and chapters on fleshy foods and eggs. The remainder of the book is taken up with a similar exhaustive study of the volatile or essential oils.

Although the book is not as inclusive as would be suggested by the title, it should be a valuable reference volume for workers in food and drug analysis. It had been the intention of the late author to cover the remaining aspects in a second volume.

---

*Clinical Medicine. Tuesday Clinics at the Johns Hopkins Hospital. By Lewellyn F. Barker, M.D., LL.D., Professor of Medicine, Emeritus, Johns Hopkins University; Visiting Physician to Johns Hopkins Hospital, Baltimore. Cloth. Price $7.00. Pp. 617. Illustrated. Philadelphia and London, W. B. Saunders Company, 1922.

†Pharmaceutical and Food Analysis. A Manual of Standard Methods for the Analysis of Oils, Fats, Waxes, and Substances in Which They Exist; Together With Allied Products. By Azor Thurston, late chemist to the Ohio State Pure Food and Drug Commission. Cloth. Pp. 416. D. Van Nostrand Co., N. Y., 1922.

*Laboratory Technique**

THIS laboratory manual is the outgrowth of the systemization of the laboratory work at St. Luke's Hospital, New York. It covers the general subjects, histology, clinical pathology, clinical analysis, bacteriology and serology as applied in the routine work of a large hospital. In the general methods, there is little new, but the short cuts and "tricks" in examination so frequently worked out in individual laboratories and so seldom considered of sufficient importance to be put into print, are incorporated and make the book highly valuable for use in routine hospital work. The authors have realized that the index will be referred to very frequently and they have therefore placed it at the beginning of the book where it supplants a table of contents. This facilitates rapid reference. The book should be of particular value to the intern and to recently graduated physicians. An occasional typographical or composing error occurs, as at the top of page 24, but this does not detract from the value of the work.

Erratum

The third paragraph on page 308 of the article by J. D. Pilcher and Torald Sollmann, February issue, should read: A control test should always be made with silver nitrate, 0.15 mg. and 0.25 mg. per 10 c.c. If 0.15 mg. should give no gas; or should 0.25 mg. give more than a trace of gas, the inhibitory dose of silver nitrate for that yeast must then be determined, and the reading for the compound corrected by the formula: True inhibiting dose of compound : determined inhibiting dose of compound : : 25 : determined inhibiting dose of silver nitrate. For example a certain sample of yeast was inhibited by 250 mg. of silvol and by 0.40 of silver nitrate.

*Laboratory Technique. The Methods Employed at St. Luke's Hospital, New York. By F. C. Wood, Karl M. Vogel and L. W. Famulener. Second edition. Paper. Price $1.35. Pp. 281. James T. Dougherty, New York. 1922.